Exhibit G

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10/15/17

Talc / Baby Powder

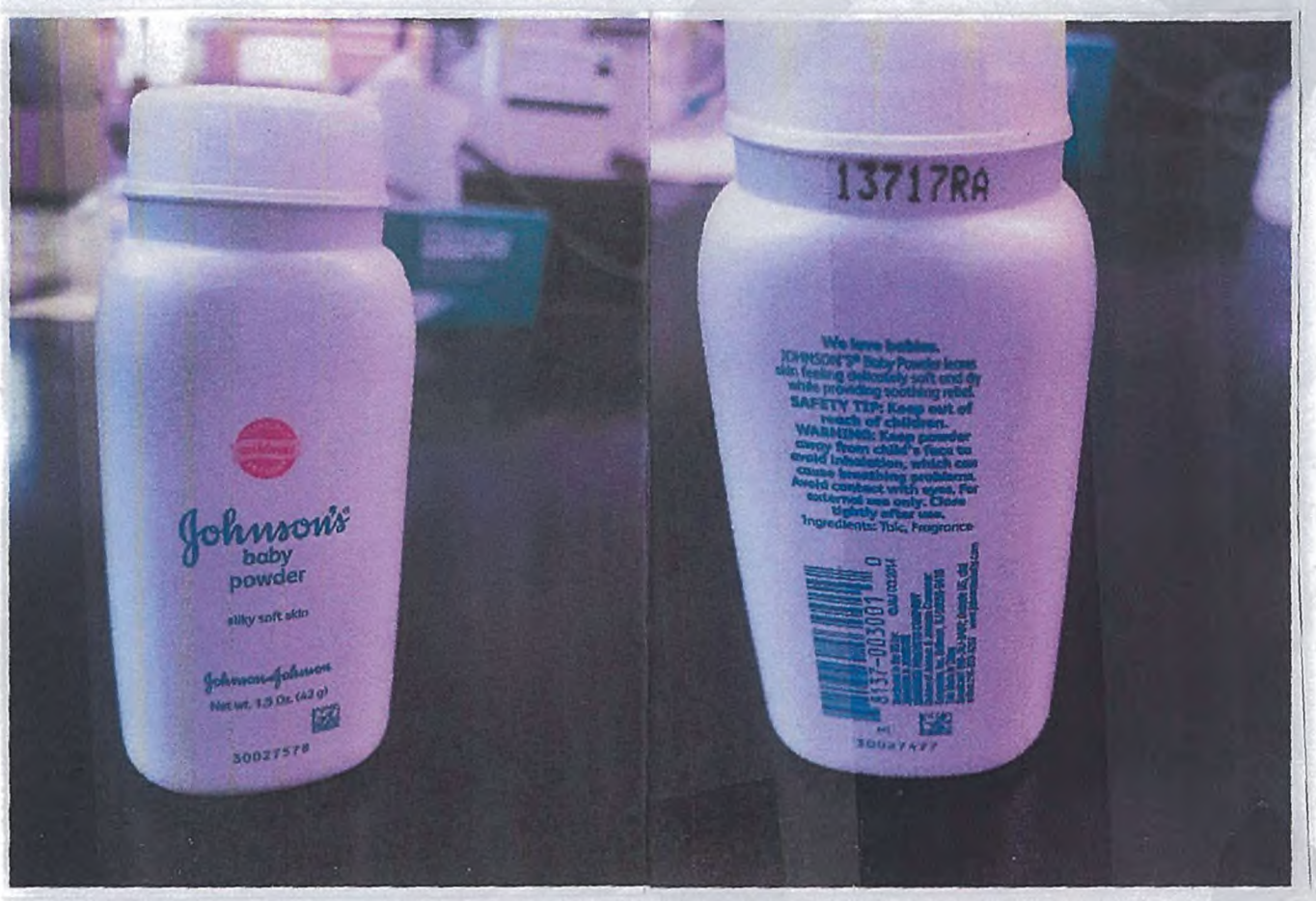
Tried to dissolve talc (Fisher # T4-500 Lot #166820) & Johnson + Johnson Baby Powder (# 30027477, Lot 13717RA)

- It won't completely dissolve - used DMSO & filtered
  - 50mg/ml

treated 200 ug/ml & 500 ug/ml in dishes

8 ul for 200 ug/ml

20 ul for 500 ug/ml


Johnson's
baby
powder
silky soft skin
Johnson & Johnson
Net wt. 1.5 Oz. (42 g)
13717RA

11/13/17

Talc / Baby Powder Treated EOC, Normal Ov. Epi, Macrophages

- Split OV90 cells, 150mm dish x2
- Split macrophages (had 4.8×10^6 cells in 150mm plate)
- Split CB. Normal Ov. epi. cells
  - had 2 x 150mm dish => ~ 11×10^6 cells
  - split into 4 plates

- Seeded TOV112-D unt. cells - treat after 24hr rest - (full plate was ~ 12×10^6 cells)

6 plates per timepoint in 100mm dish

ID	treatment	details
266	TOV112 - unt - 24hr	2×10^6 cells
267	Control (DMSO) - 24h	"
268	500 µg/mL Talc - 24h	"
269	1000 µg/mL Talc - 24h	"
270	500 µg/mL Baby Powder (BP) - 24h	"
271	1000 µg/mL BP - 24h	"
272	TOV112 - unt 48hr	1×10^6 cells
273	Control 48h	"
274	500 µg/mL Talc 48h	"
275	1000 µg/mL Talc 48h	"
276	500 µg/mL B.P. 48h	"
277	1000 µg/mL [illegible]	"
278	TOV1120 - unt 72 hr	500,000 cells
279	Control - 72hr	"
280	500 µg/mL Talc - 72h	"
281	1000 µg/mL Talc - 72h	"
282	500 µg/mL B.P. 72h	"
283	1000 µg/mL B.P. 72hr	"

from pg 2

11/14/17

Treat cells after 24 hr rest

$(x)(50{,}000\ \mu g/ml) = (10\ ml)(500\ \mu g/ml)$

$x = 100\ \mu l$

For 1000 µg/ml = 200 µl

Controls get 200ul of sterile Dmso

- made a master mix of media + treatment and then added it to the cells

500 µg/ml = 300µl + 30 ml

1000 µg/ml = 600µl + 30 ml

Dmso Control = 600ul + 30 ml

ID	Treatment
284	OV90 Untreatd 24 hours
285	24 hr DMSO Control
286	24 hr 500 ug/ml Talc
287	25 hr 1000 ug/ml Talc
288	24 hr 500 ug/ml Baby Powder
289	24 hr 1000 ug/ml Baby Powder
290	Ov90 Untreatd 48 hours
291	48 hr DMSO Control
292	48 hr 500 ug/ml Talc
293	48 hr 1000 ug/ml Talc
294	48 hr 500 ug/ml Baby Powder
295	48 hr 1000 ug/ml Baby Powder
296	Ov90 72 hr untreated
297	72 hr DMSO Control
298	72 hr 500 ug/ml Talc
299	72 hr 1000 ug/ml Talc
300	72 hr 500 ug/ml Baby Powder
301	72 hr 1000 ug/ml Baby Powder
302	EL1 Untreatd 24 hours
303	24 hr DMSO Control
304	24 hr 500 ug/ml Talc
305	25 hr 1000 ug/ml Talc
306	24 hr 500 ug/ml Baby Powder
307	24 hr 1000 ug/ml Baby Powder
308	EL1 Untreatd 48 hours
309	48 hr DMSO Control
310	48 hr 500 ug/ml Talc
311	48 hr 1000 ug/ml Talc
312	48 hr 500 ug/ml Baby Powder
313	48 hr 1000 ug/ml Baby Powder
314	EL1 72 hr untreated
315	72 hr DMSO Control
316	72 hr 500 ug/ml Talc
317	72 hr 1000 ug/ml Talc
318	72 hr 500 ug/ml Baby Powder
319	72 hr 1000 ug/ml Baby Powder

ID	Treatment
320	TOV-21G Untreatd 24 hours
321	24 hr DMSO Control
322	24 hr 500 ug/ml Talc
323	25 hr 1000 ug/ml Talc
324	24 hr 500 ug/ml Baby Powder
325	24 hr 1000 ug/ml Baby Powder
326	TOV-21G Untreatd 48 hours
327	48 hr DMSO Control
328	48 hr 500 ug/ml Talc
329	48 hr 1000 ug/ml Talc
330	48 hr 500 ug/ml Baby Powder
331	48 hr 1000 ug/ml Baby Powder
332	TOV-21G 72 hr untreated
333	72 hr DMSO Control
334	72 hr 500 ug/ml Talc
335	72 hr 1000 ug/ml Talc
336	72 hr 500 ug/ml Baby Powder
337	72 hr 1000 ug/ml Baby Powder
338	Cell Biologics - Normal Ovarian Epithelial cells, Unt 24 hrs
339	24 hr DMSO Control
340	24 hr 500 ug/ml Talc
341	25 hr 1000 ug/ml Talc
342	24 hr 500 ug/ml Baby Powder
343	24 hr 1000 ug/ml Baby Powder
344	Cell Biologics - Normal Ovarian Epithelial cells, Unt 48 hrs
345	48 hr DMSO Control
346	48 hr 500 ug/ml Talc
347	48 hr 1000 ug/ml Talc
348	48 hr 500 ug/ml Baby Powder
349	48 hr 1000 ug/ml Baby Powder
350	Cell Biologics - Normal Ovarian Epithelial cells, Unt 72 hrs
351	72 hr DMSO Control
352	72 hr 500 ug/ml Talc
353	72 hr 1000 ug/ml Talc
354	72 hr 500 ug/ml Baby Powder
355	72 hr 1000 ug/ml Baby Powder

11/17/17

seeded C.B. Normal Ov. Epi Cells

338-355 - passage 11

24h = 2x10^6 cells
48h = 1x10^6 cells
72h = 500,000 cells

Will treat on 11/19/17
*collected 24hr 11/20/17
48hr 11/21/17
72h 11/22/17

11/19/17 seeded EL-1, treated on 11/19/17
for 24hr 4x10^6 collect 11/20/17
48hr 2x10^6 collect 11/21/17
72hr 1x10^6 collect 11/22/17

12/4/17 Seeded TOV1120 / TOV21G / treat 12/5/17
Collect 24hr 12/6/17
" 48hr 12/7/17
" 72hr 12/8/17

12/7/17

RNA extraction

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230
1	267	12/7/2017 2:07:20 PM	0.0635	µg/µl	1.588	0.831	1.91	1.36
2	269	12/7/2017 2:07:45 PM	0.0548	µg/µl	1.370	0.695	1.97	0.29
3	273	12/7/2017 2:08:08 PM	0.0257	µg/µl	0.643	0.335	1.92	0.24
4	275	12/7/2017 2:08:29 PM	0.0164	µg/µl	0.409	0.212	1.93	0.66
5	285	12/7/2017 2:08:53 PM	0.0678	µg/µl	1.694	0.882	1.92	1.13
6	287	12/7/2017 2:09:13 PM	0.0553	µg/µl	1.381	0.722	1.91	2.32
7	291	12/7/2017 2:09:32 PM	0.0630	µg/µl	1.575	0.802	1.96	0.34
8	293	12/7/2017 2:09:51 PM	0.0506	µg/µl	1.265	0.648	1.95	1.39
9	297	12/7/2017 2:10:10 PM	0.0358	µg/µl	0.896	0.455	1.97	0.22
10	299	12/7/2017 2:10:30 PM	0.0248	µg/µl	0.621	0.313	1.99	0.86
11	303	12/7/2017 2:10:51 PM	0.1809	µg/µl	4.523	2.334	1.94	1.35
12	305	12/7/2017 2:11:10 PM	0.1508	µg/µl	3.770	1.925	1.96	1.75
13	309	12/7/2017 2:11:31 PM	0.0279	µg/µl	0.698	0.362	1.93	0.85
14	311	12/7/2017 2:11:53 PM	0.0675	µg/µl	1.688	0.877	1.92	0.35
15	315	12/7/2017 2:12:12 PM	0.0445	µg/µl	1.113	0.585	1.90	1.13
16	317	12/7/2017 2:12:31 PM	0.0587	µg/µl	1.468	0.770	1.91	0.60
17	321	12/7/2017 2:12:50 PM	0.0810	µg/µl	2.025	1.061	1.91	1.03
18	323	12/7/2017 2:13:10 PM	0.0326	µg/µl	0.815	0.408	2.00	1.00
19	327	12/7/2017 2:13:31 PM	0.0445	µg/µl	1.112	0.574	1.94	2.54
20	329	12/7/2017 2:14:02 PM	0.0092	µg/µl	0.230	0.114	2.02	0.10
21	339	12/7/2017 2:14:21 PM	0.0177	µg/µl	0.442	0.220	2.01	0.55
22	341	12/7/2017 2:14:40 PM	0.0172	µg/µl	0.429	0.221	1.94	0.89
23	345	12/7/2017 2:14:59 PM	0.0219	µg/µl	0.548	0.281	1.95	1.31
24	347	12/7/2017 2:15:17 PM	0.0165	µg/µl	0.414	0.207	2.00	0.56
25	351	12/7/2017 2:15:34 PM	0.0165	µg/µl	0.413	0.214	1.93	0.96
26	353	12/7/2017 2:15:52 PM	0.0112	µg/µl	0.281	0.142	1.98	0.94
27	279	12/8/2017 1:19:05 PM	0.0145	µg/µl	0.364	0.192	1.89	1.07
28	281	12/8/2017 1:19:28 PM	0.0089	µg/µl	0.222	0.111	2.00	0.48
29	333	12/8/2017 1:19:56 PM	0.0244	µg/µl	0.609	0.317	1.92	0.65
30	335	12/8/2017 1:20:15 PM	0.0039	µg/µl	0.097	0.054	1.79	0.39
31	335	12/8/2017 1:21:01 PM	0.0041	µg/µl	0.102	0.048	2.12	0.38

12/8/17

(VILO) cDNA synthesis
0.1 µg RNA used except
fw #335 (0.06 µg)

Sample ID	ul RNA for 0.1 ug rxn	ul Water
267	1.6	14.4
269	1.8	14.2
273	3.9	12.1
275	6.1	9.9
279	6.9	9.1
281	11.2	4.8
285	1.5	14.5
287	1.8	14.2
291	1.6	14.4
293	2.0	14.0
297	2.8	13.2
299	4.0	12.0
303	0.6	15.4
305	0.7	15.3
309	3.6	12.4
311	1.5	14.5
315	2.2	13.8
317	1.7	14.3
321	1.2	14.8
323	3.1	12.9
327	2.2	13.8
329	10.9	5.1
333	4.1	11.9
335	16.0	0.0
339	5.6	10.4
341	5.8	10.2
345	4.6	11.4
347	6.1	9.9
351	6.1	9.9
353	8.9	7.1

1/10/18 CA-125 ELISA

RayBio # ELH-CA125

Test uncencentrated media vs concentrated using Amecon Ultra-15 filter MW cutoff 10,000

Weigh tubes	Volume	~~empty~~	empty sample reservoir ~~w/ media~~	empty centrifuge tube	media + reservoir
266	8.36mL	~~25.0 g~~	10.99g	11.59g	19.17g
338	8.853mL	~~24.9 g~~	10.98g	11.53g	19.7g

Spin tubes for 25 min at 4000xg

move the retentate by pipetting into new container

weigh retentate	tube weight	tube + retentate	filtrate
266	1.0025g	1.3514g	19.29g
338	1.0088g	1.7104g	19.4797g

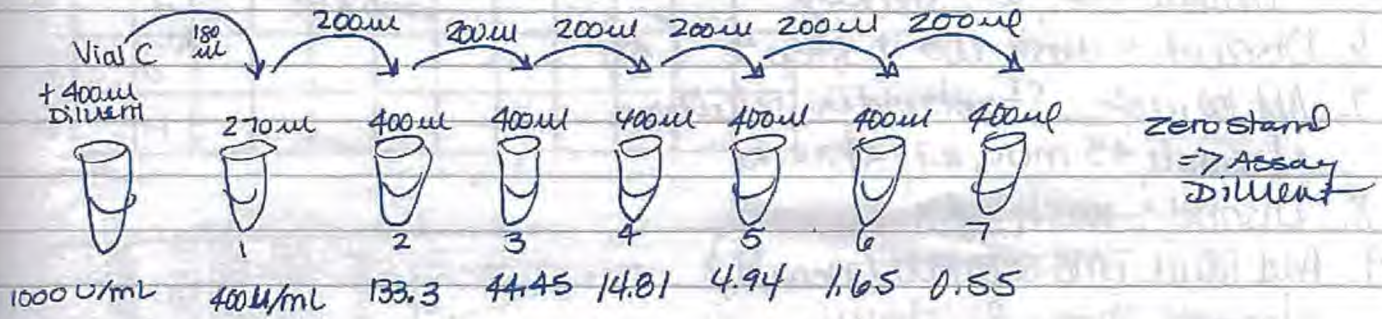
$$\% \text{ retentate recovery} = 100 \times \frac{W_r \times C_r}{W_o \times C_o}$$

$$\% \text{ filtrate recovery} = 100 \times \frac{W_p \times C_f}{W_o \times C_o}$$

$$\% \text{ recovery} = \% \text{ retentate recovery} + \% \text{ filtrate recovery}$$

1/10/18 CA-125 EU84

1. All reagents & samples to room temperature
2. Assay diluent (Item E2) should be diluted 5x w/ dd H_2O
   - Stable 1mo. at 4°C
3. Prep. of Standard
   - Spin vial C
   - Add 400µl 1x Assay Diluent into vial C = 1000 U/mL
     - mix gently



4. Prepare Wash buffer by diluting 20x
   - Stable 1mo. at 4°C

5. Spin Item F, detection antibody
   - Add 100 µl of 1x Assay Diluent
     - Stable 5days at 4°C
   - Dilute it ~~800x~~ 80x (1ml assay diluent) and will be used in assay

6. Spin HRP-Strep. Vial (Item G) & mix
   - Add 15µl to tube w/ 12mL of assay diluent (800x)
     - do not save!

Go to pg 10

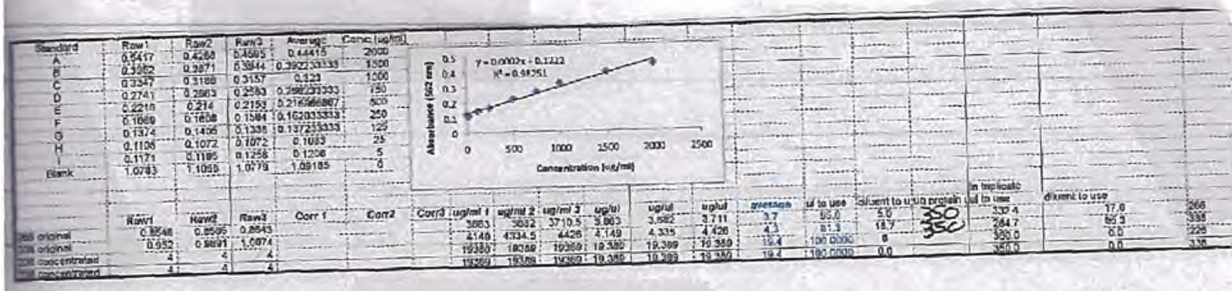
from pg 9

Assay Procedure

1. All samples to RT.
2. Label 8 strip/wells
3. Add 100µl standard / samples
   - Incubate 2.5 hr, gentle shaking, RT
4. Discard solution, wash 4x
   - decant, blot after washes
5. Add 100µl of 1X Antibody mix
   - Incubate 1 hr, RT, shaking
6. Discard & wash as in step 4 (4x)
7. Add 100µl of Streptavidin solution
   - Incubate 45 min, RT, shaking
8. Discard & wash 4X
9. Add 100µl TMB substrate (Item H)
   - Incubate 30 min, RT, shaking
10. Add 50µl STOP solution (Item I) to each well
    - Read at 450nm

1/10/18 Protein levels in media for CA125 Assay

	1	2	3	4	5	6	7	8	9	10	11	12
A	Stand	A		Stand		I						
B		B		Blank								
C		C		266 orig								
D		D		338 orig								
E		E		266 conc.								
F		F		338 conc.								
G		G										
H		H										



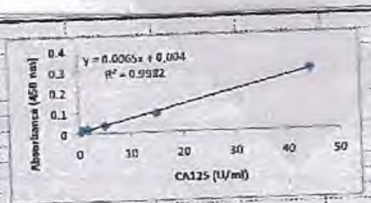
1/11/18

CA125 ELISA - test levels in media

	1	2	3	4	5	6	7	8	9	10	11	12
A	Stand	1		266 orig								
B		2		338 orig.								
C		3		266 conc								
D		4		338 conc								
E		5		media blank								
F		6										
G		7										
H	Blank											

1/11/18
Test media amounts for CA125 ELISA

Standard (U/ml)	OD1 (450 nm	OD1		Corrected OD1	Corrected OD2	Average
400	2.3656	2.3921		2.31885	2.32545	2.3222
133.3	1.1825	1.1458		1.11585	1.07915	1.0975
44.45	0.3643	0.358		0.29765	0.29135	0.2945
14.81	0.1593	0.1562		0.09265	0.08955	0.0911
4.94	0.1049	0.1009		0.03825	0.03425	0.03625
1.65	0.082	0.0861		0.01535	0.01945	0.0174
0.55	0.0769	0.0776		0.01025	0.01095	0.0106
Blank	0.0661	0.0672		0.00665		



Samples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	Corrected for Media OD1	Corrected for Media OD2	Corrected for Media OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation
Original 266	0.098	0.0925	0.0939	0.02935	0.02585	0.02725	0.0155	0.012	0.0134	1.769230769	1.230769231	1.446153846	1.482051282	0.271019096
Original 338	0.1179	0.1155	0.1172	0.06125	0.04885	0.05055	0.0374	0.035	0.0367	5.138461538	4.769230769	5.030769231	4.979487179	0.189882139
Concentrated 266	0.0649	0.0799	0.0843	0.01825	0.01325	0.01765	0.0044	-0.0006	0.0038	0.061538462	-0.70769231	-0.03076923	-0.22564103	0.42001221
Concentrated 338	0.2216	0.2312	0.2172	0.15495	0.15455	0.15055	0.1411	0.1507	0.1367	21.09230769	22.56923077	20.41538462	21.35897436	1.101406667
Plain Media	0.0769	0.0885	0.0811	0.01325	0.02185	0.01445	0.01385							

266 = TOV1120 cell

338 = Normal Ov. Epi cells

Proceed using unconcentrated media

1/12/18

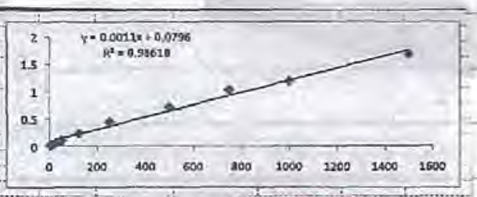
Protein levels for CA125 assay

Re-did standard and original media - media was too concentrated

- diluted media by 50%, remeasured
- Also used 25 µl of the standard and samples

1/12/18 Using 25 ul of standard

Standard	Raw1	Raw2	Raw3	Corr1	Corr2	Corr3	Average	Conc.(ug/ml)
B1	1.8393	1.7711	1.7805	1.736933333	1.68783333	1.678	1.673433333	1500
C1	1.277	1.2876	1.2926	1.174633333	1.18523333	1.19	1.183366667	1000
D1	1.1027	1.1374	1.1232	1.000333333	1.03503333	1.021	1.018733333	750
E1	0.8031	0.8012	0.8102	0.700733333	0.69883333	0.706	0.702466667	500
A	0.5556	0.527	0.5416	0.453233333	0.42463333	0.439	0.439	250
B	0.336	0.311	0.3407	0.239633333	0.20863333	0.238	0.228866667	125
C	0.2049	0.1908	0.2023	0.102533333	0.08843333	0.1	0.096966667	50
D	0.157	0.1555	0.154	0.054633333	0.05313333	0.052	0.053133333	25
E	0.118	0.1119	0.1112	0.015633333	0.00953333	0.009	0.009183333	5
Blank	0.1036	0.1005	0.1027	0.102366667				0



Samples	Raw 1	Raw 2	Raw 3	Corr 1	Corr 2	Corr 3	ug/ml 1	ug/ml 2	ug/ml 3	Avg	x df	ul sample (sg to use per well = 350)	ul diluent
279	2.2288	2.2411	2.2644	2.1337	2.146	2.169	1.867363636	1.87654545	1.89673	1.88188	3.76376768	325.5	24.5
281	2.4279	2.388	2.3488	2.3328	2.2709	2.252	2.048363636	1.99209091	1.97464	2.00503	4.01006061	305.5	44.5
297	3.5204	3.4285	3.3984	3.4253	3.3334	3.301	3.041454545	2.956	2.92682	2.97612	5.95224242	205.8	144.2
299	3.3355	3.3228	3.4021	3.2404	3.2278	3.307	2.873454545	2.862	2.934	2.88982	5.77963636	212.0	138.0
333	2.7243	2.5049	2.2334	2.6292	2.4098	2.138	2.317818182	2.11636364	1.87155	2.10258	4.20515152	291.3	58.7
335	2.2042	2.4767	2.3071	2.1091	2.3836	2.212	1.845	2.09454545	1.93855	1.95936	3.91872727	312.8	37.4
351	3.0495	3.0067	2.9638	2.9544	2.9116	2.869	2.613454545	2.57454545	2.53555	2.57452	5.1490303	237.9	112.1
353	2.8432	2.768	3.0064	2.7481	2.6729	2.911	2.425909091	2.35754545	2.57427	2.45258	4.90515152	249.7	100.3
Blank	0.0944	0.0981	0.0928	0.0961									

1/12/2018

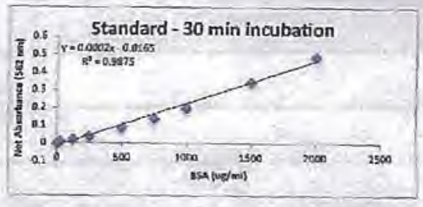
Samples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation
TOV112D 72 hr control	279	0.104	0.0885	0.0969	0.03735	0.02185	0.03025	5.13076923	2.74615385	4.03846154	4.58461538	0.77237818
TOV112D 72 hr 1000 ug/ml Talc	281	0.0806	0.0742	0.0838	0.01395	0.00755	0.01715	1.53076923	0.54615385	2.02307692	1.77692308	0.34811411
OV90 72 hr control	297	0.1059	0.1046	0.1321	0.03925	0.03795	0.08545	5.42307692	5.22307692	9.45384615	5.32307692	0.14142136
Ov90 72 hr 1000 ug/ml Talc	299	0.1062	0.0906	0.1316	0.03955	0.02395	0.06495	5.46923077	3.06923077	9.37692308	4.26923077	1.69705627
TOV-21G 72 hr control	333	0.0724	0.0716	0.0942	0.00575	0.00495	0.02755	0.26923077	0.14615385	3.62307692	0.20769231	0.08702853
TOV-21G 72 hr 1000 ug/ml Talc	335	0.0761	0.077	0.0956	0.00945	0.01035	0.01585	0.83846154	0.97692308	2.3	0.90769231	0.09790709
Normal Ov Epithelial 72 hr control	351	0.1003	0.0943	0.1001	0.03365	0.02765	0.03345	4.56153846	3.53846154	4.53076923	4.54615385	0.02175713
Normal Ov Epithelial 72 hr 1000 ug/ml talc	353	0.1106	0.092	0.1331	0.04395	0.02535	0.06645	6.14615385	3.28461538	9.60769231	4.71538462	2.02341325
b		0.0661	0.0672		0.06665							

The other proteins in media may be interfering. Try lysate.

Protein levels in lysate

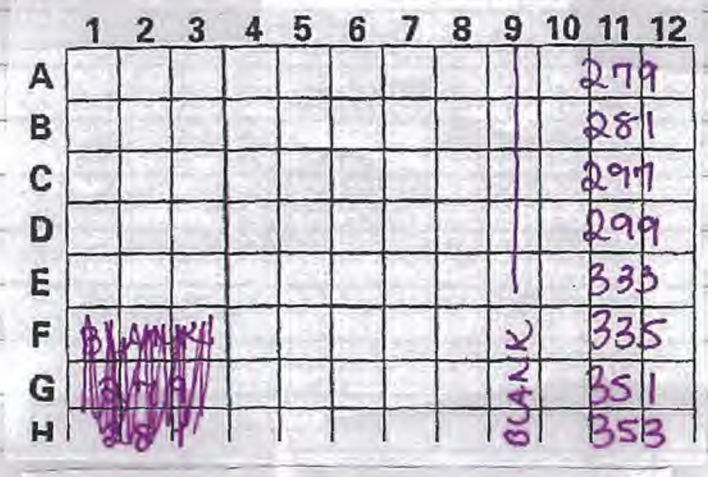
1-16-18

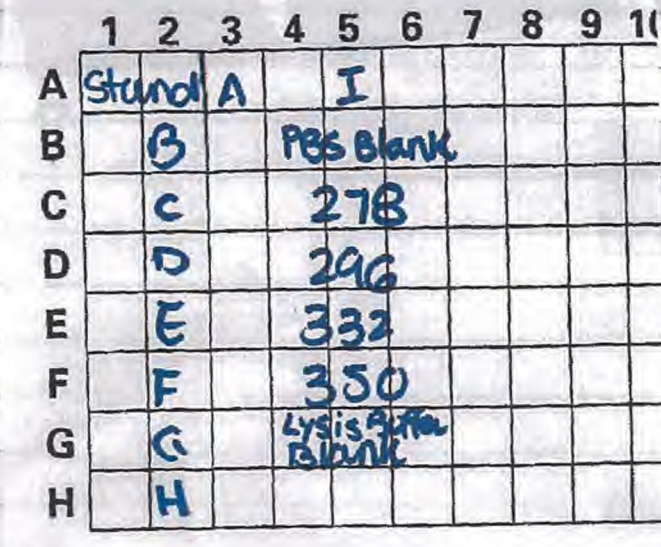
Standard ID	Concentration (ug/ml)	OD1	OD2	OD3	Average	Corrected Avg
A	2000	0.5869	0.5848	0.5582	0.57596667	0.4874
B	1500	0.4457	0.4211	0.3533	0.4334	0.34483333
C	1000	0.3213	0.2774	0.2593	0.286	0.19743333
D	750	0.2473	0.2199	0.2128	0.22866667	0.1301
E	500	0.181	0.1759	0.1713	0.17606667	0.0875
F	250	0.1328	0.1252	0.1256	0.12786667	0.0393
G	125	0.1105	0.112	0.1116	0.11136667	0.0228
H	25	0.1004	0.0941	0.0953	0.0966	0.00803333
I	5	0.0845	0.0840	0.0941	0.08773333	0
J (BLANK)	0	0.0881	0.0884	0.0892	0.08856667	0



	ID	OD1	OD2	OD3	Corr OD1	Corr OD2	Corr OD3	ug/ul 1	ug/ul 2	ug/ul 3	Average	using 100 ug per well	x 3.5 wells	dilution
TOV112D 72 hr control	279	0.3429	0.2301	0.2326	0.1356	0.1226	0.1253	7.606	6.965	7.09	7.0275	14.22	49.8	300.2
TOV112D 72 hr 1000 ug/ml Talc	281	0.1892	0.1923	0.1878	0.0819	0.085	0.0805	4.92	5.075	4.85	4.94833333	20.21	70.7	279.3
OV90 72 hr control	297	0.3198	0.331	0.3306	0.2125	0.2237	0.2233	11.45	12.01	11.99	12	8.33	29.2	320.5
Ov90 72 hr 1000 ug/ml Talc	299	0.2689	0.2759	0.2784	0.1616	0.1686	0.1711	8.905	9.255	9.36	9.3175	10.73	37.6	312.4
TOV-21G 72 hr control	333	0.2991	0.2968	0.2948	0.1918	0.1895	0.1875	10.415	10.3	10.2	10.305	9.70	34.0	316.0
TOV-21G 72 hr 1000 ug/ml Talc	335	0.1668	0.1616	0.1634	0.0595	0.0543	0.0551	3.75	3.54	3.63	3.64	27.47	96.2	253.8
Normal Ov Epithelial 72 hr control	351	0.2359	0.23	0.23	0.1286	0.1227	0.1227	7.255	6.98	6.96	6.96	14.37	50.3	299.7
Normal Ov Epithelial 72 hr 1000 ug/ml talc	353	0.2432	0.2474	0.2344	0.1359	0.1401	0.1271	7.82	7.83	7.18	7.725	12.94	45.3	304.7
b		0.1097	0.1049		0.1073									

	ID	OD1	OD2	OD3	Corr OD1	Corr OD2	Corr OD3	ug/ul 1	ug/ul 2	ug/ul 3	Average	using 100 ug per well	x 3.5 wells	dilution
TOV112D 72 hr unt	278	0.6686	0.6925	0.653	0.56466667	0.58876667	0.54926667	29.0683333	30.2633333	28.2883333	29.2733333	3.42	12.0	338.0
Ov90 72 hr unt 72 hr	296	0.34	0.3407	0.3386	0.23626667	0.23696667	0.23286667	12.5383333	12.5733333	12.4683333	12.5933333	7.94	27.8	322.2
TOV-121G unt	332	0.4392	0.4456	0.4413	0.33546667	0.34186667	0.33756667	17.5983333	17.9183333	17.7033333	17.7400000	5.61	19.7	330.3
Normal Ov Epithelial 72 hr unt	350	0.2281	0.2347	0.2316	0.12436667	0.13096667	0.12786667	7.04333333	7.37333333	7.21833333	7.29583333	13.71	48.0	302.0
b		0.1032	0.104	0.104	0.10373333									





1-17-18

CA125 in Lysate

	1	2	3	4	5	6	7	8	9	10	11	12
A	279											
B	281											
C	297											
D	299											
E	333											
F	335											
G	351			BLANK								
H	353											

	1	2	3	4	5	6	7	8	9	10	11	12
A											278	
B											278	
C											296	
D											296	
E											332	
F											332	B
G											350	332
H											350	350

1/17/2018

Lysate

Samples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation
279	0.1637	0.1749	0.171	0.0907	0.0919	0.088	11.8	13.5230769	12.9230769	13.2230769	0.42426407
281	0.1188	0.1175	0.1221	0.0358	0.0345	0.0391	4.89230769	4.69230769	5.4	4.99487179	0.36482413
297	0.146	0.1479	0.1689	0.063	0.0649	0.0859	9.07692308	9.36923077	12.6	9.22307692	0.20669275
299	0.1371	0.13	0.1506	0.0541	0.047	0.0676	7.70769231	6.61538462	9.78461538	7.16153846	0.77237618
333	0.1374	0.1269	0.1269	0.0544	0.0439	0.0459	7.75384615	6.13846154	6.44615385	6.29230769	0.21757132
335	0.1	0.0889	0.0907	0.017	0.0059	0.0077	2	0.29230769	0.56923077	0.43076923	0.19581419
351	0.1491	0.1518	0.1547	0.0661	0.0686	0.0717	9.55384615	9.90923077	10.4153846	9.76153846	0.28372128
353	0.1551	0.1362	0.1467	0.0721	0.0532	0.0637	10.4769231	7.58923077	9.18461538	9.83076923	0.91379853
	0.0868	0.0876	0.0746	0.093							

b

1/18/2018

Lysate

Samples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	Standard Deviation
278	0.1998	0.1904		0	0.0476	0.0382	5.70769231	5.26153846		5.98461538	1.02258519
296	0.1976	0.1971		0.199	0.0456	0.0449	6.4	6.29230769		6.34615385	0.07614996
332	0.1875	0.179		0.2328	0.0353	0.0266	4.81538462	3.50769231	6.58461538	4.16153846	0.9246781
350	0.2533	0.2874			0.1011	0.1352	14.9384615	20.1846154	11.7846154	13.3615385	2.230106
	0.1522										

b

Results: Lysate protein measurements may be affected by talc.
Repeat protein measurements, have control of talc in it.

Remeasured protein levels in media 1-19-18

- Recalc. data. CA125

Re-measure media protein

1-19-18

	1	2	3	4	5	6	7	8	9	10	11	12
A				BLANK Media 10x		279						
B						281						
C						297						
D						299						
E				Biotin XBS		333						
F						335						
G						351						
H						353						

- 10x diluted samples media was 10x as blank
- used ZSup to detect

protein → New control

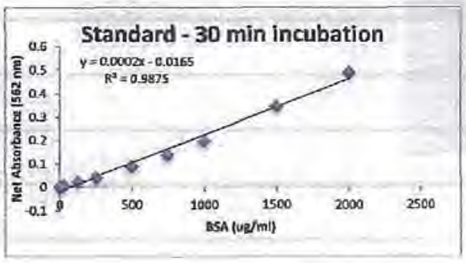
	1	2	3	4	5	6	7	8	9	10	11	12
A							278			335		
B							279			350		
C							281			351		
D							296			353		
E							297			Lysis Buffer		
F							299			Lysis Buffer + talc		
G							332					
H							333					

44 x 200 = 8800 / 50 = 176

Go to pg 17

from pg 16

Standard ID	Concentration (ug/ml)	OD1	OD2	OD3	Average	Corrected Avg
A	2000	0.5869	0.5848	0.5582	0.576967	0.4874
B	1500	0.4457	0.4211	0.3533	0.4334	0.344833
C	1000	0.3213	0.2774	0.2593	0.286	0.197433
D	750	0.2473	0.2199	0.2128	0.226667	0.1381
E	500	0.181	0.1759	0.1713	0.176067	0.0875
F	250	0.1328	0.1252	0.1258	0.127867	0.0393
G	125	0.1105	0.112	0.1116	0.111367	0.0228
H	25	0.1004	0.0941	0.0953	0.0966	0.008033
I	5	0.0845	0.0846	0.0941	0.087733	0
J (BLANK)	0	0.0881	0.0884	0.0892	0.088567	0



	ID	OD1	OD2	OD3	Corr OD1	Corr OD2	Corr OD3	ug/ul 1	ug/ul 2	ug/ul 3	Average	how much was used	ug used
TOV112D 72 hr unt	278	0.6692	0.6792	0.6667	0.559667	0.6792	0.6667	28.81833	34.785	34.16	34.4725	3.42	117.9
TOV112D 72 hr control	279	0.2121	0.2206	0.2113	0.102767	0.2206	0.2113	5.963333	11.855	11.39	11.6225	14.23	165.4
TOV112D 72 hr 1000 ug/ml Talc	281	0.1866	0.1902	0.189	0.077267	0.1902	0.189	4.888333	10.335	10.275	8.432778	20.21	170.4
Ov90 72 hr unt 72 hr	296	0.3372	0.3405	0.3354	0.227867	0.3405	0.3354	12.21833	17.85	17.595	15.88778	7.94	126.1
OV90 72 hr control	297	0.3217	0.3231	0.3255	0.212367	0.3231	0.3255	11.44333	16.98	17.1	17.04	8.33	141.9
Ov90 72 hr 1000 ug/ml Talc	299	0.2813	0.282	0.2785	0.171967	0.282	0.2785	9.423333	14.925	14.75	14.8375	10.73	159.2
TOV-121G unt	332	0.4161	0.4393	0.452	0.306767	0.4393	0.452	16.16333	22.79	23.425	23.1075	5.61	129.6
TOV-21G 72 hr control	333	0.3031	0.3039	0.3238	0.193767	0.3039	0.3238	10.51333	16.02	17.015	14.51611	9.70	140.8
TOV-21G 72 hr 1000 ug/ml Talc	335	0.1847	0.1856	0.19	0.075367	0.1856	0.19	4.593333	10.105	10.325	8.341111	27.47	229.1
Normal Ov Epithelial 72 hr unt	350	0.2227	0.2338	0.2367	0.113367	0.2338	0.2367	6.493333	12.515	12.66	12.5875	13.71	172.6
Normal Ov Epithelial 72 hr control	351	0.2287	0.2316	0.2402	0.119367	0.2316	0.2402	6.793333	12.405	12.835	12.62	14.37	181.3
Normal Ov Epithelial 72 hr 1000 ug/ml talc	353	0.2277	0.2391	0.2432	0.116367	0.2391	0.2432	6.743333	12.78	12.985	9.761667	12.94	126.3
Lysis buffer blank		0.1102	0.1063	0.1095	0.109333								
Lysis buffer+talc blank		0.1073	0.1068	0.1059	-0.00203	-0.00253	-0.00343	-0.00267					

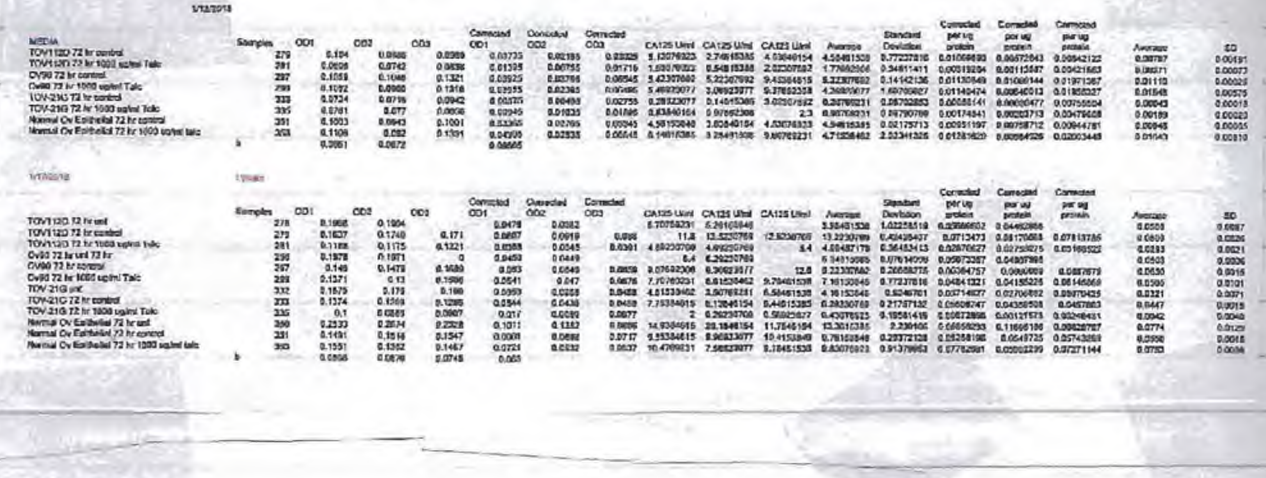
Remeasured Media Protein - 10x diluted using 25ul standard

Samples		Raw 1	Raw 2	Raw 3	Corr 1	Corr 2	Corr 3	ug/ul 1	ug/ml 2	ug/ml 3	Avg	xdf	ul media used	actual ug used
	279	0.7155	0.7569	0.7571	0.6192	0.6606	0.6608	0.490545	0.528182	0.528364	0.515697	5.15697	92.992174	479.5678
	281	0.7067	0.7139	0.7356	0.6104	0.6176	0.6393	0.482545	0.489091	0.508818	0.493485	4.934848	87.280476	430.7159
	297	1.1257	1.1418	1.1656	1.0294	1.0455	1.0693	0.863455	0.878091	0.899727	0.880424	8.804242	58.801368	517.7015
	299	1.1303	1.1325	1.1642	1.034	1.0362	1.0679	0.867636	0.869636	0.916636	0.884636	8.846364	60.557443	535.7132
	333	0.7006	0.7217	0.7401	0.6043	0.6254	0.6438	0.477	0.496182	0.512909	0.495364	4.953636	83.231246	412.2973
	335	0.7315	0.7361	0.7684	0.6352	0.6398	0.6721	0.505091	0.509273	0.538636	0.517667	5.176667	89.314713	462.3525
	351	0.9217	0.9433	0.9655	0.8254	0.847	0.8692	0.676	0.697636	0.717818	0.697818	6.978182	67.973964	474.3347
	353	0.9401	0.9952	1.0083	0.8438	0.8989	0.912	0.694727	0.744818	0.756727	0.732091	7.320909	71.353555	522.3729
Blank-10x media		0.7339	0.7117	0.7024	0.6376	0.7117	0.7024	0.70705						
Blank-PBS		0.0941	0.0998	0.095	0.0963									

Recalculated how much protein was really used and then adjusted CA125 levels pg 18

from pg 17

CA125 - recalc. for SRI abstract



	fold increase	fold increase	fold increase	Average	SD
OV90	1.02745665	0.5765896	1.76156069	1.39450867	0.51908995
TOV 21G	4.03703704	4.7037037	11.0740741	4.37037037	0.47140452
↳ Normal Ov Epi	1.35194585	0.72250423	2.11336717	1.73265651	0.53840618

Media calculations for OV90, 21G, Normal Ov. Epi were used for SRI abstract

1/29/18

Seed 72 hour 1000 ug/ml talc EOC

SKOV-3 and A2780 seeded 4x10^6 cells
TOV-21G seeded 2x10^6 cells

Treat SKOV-3 and A2780 today 1/29/18
25mL of media

(x)(100mg/ml) = (25ml)(1000 ug/ml)
x = 250 uL

Treat TOV21G 1/30/18

1/31/18 - The presence of 1000 ug/ml is physically killing the cells.
- We need to decrease dose.

~~4/3~~ 2/1/18

Treat EOC w/talc

- Needed to lower the dose of talc (in PBS saline)
  - unt, 5, 20, 100 ug/mL → 72 hours

- Seeded 1x10^6 cells — 60mm dish, 5mL

		ID
- EL1	unt	356
	EL1 5ug/mL	357
	EL1 20ug/mL	358
	EL1 100ug/mL	359
- SKov-3	unt	360
	5ug/mL	361
	20ug/mL	362
	100ug/mL	363
- TOV1120	unt	364
	5 ug/mL	365
	20 ug/mL	366
	100 ug/mL	367
- A2780	unt	368
	5 ug/mL	369
	20 ug/mL	370
	100 ug/mL	371
- ~~OV90~~	~~unt~~	~~372~~
	~~5 ug/mL~~	~~373~~
	~~20 ug/mL~~	~~374~~
	~~100 ug/mL~~	~~375~~

		ID
EL1	Control 5	~~379~~
	Control 1000	~~380~~
	1000 ug/mL talc	~~381~~
SKov-3	Control 5	~~382~~
	Control 1000	~~383~~
	1000 ug/mL talc	~~384~~
A2780	Control 5	385
	Control 1000	~~386~~
	1000 ug/mL talc	~~387~~
Normal OV Epi	unt	~~388~~ 383
5	5 ug/mL	~~389~~ 384
20	20	~~390~~ 385
100	100	~~391~~ OK 386
	1000	~~392~~
	Control 5	~~393~~
	Control 1000	~~394~~
TOV1120	Control 5	BGS
	Control 1000	396
	1000 ug/mL talc	~~397~~

FT 33

~~No~~	unt 379	
	5ug/mL 380	
	20 381	OK
	100 382	
	~~1000 383~~	
	~~Control 5 377~~	
	~~Control 1000 378~~	

2/2/18

Prepare talc

100 mg in 10mL → mix and filter = 10 mg/mL

2/2/18 treated w/ talc

(x)(10000 ug/mL) = (5mL)(5 ug/mL) = 2.5 mL

(x)(10000 ug/mL) = (5mL)(20 ug/mL) = 10 uL

(x)(10000 ug/mL) = (5mL)(100 ug/mL) = 50 uL

2/6/18

treat w/ "soaked" talc (10000 ug/mL)

* talc was rocked for 72 hours, spun and supernatant collected

★ too much volume

· made 1g/10 mL DMSO - re-soak 72hrs

2/7/18 seeded Normal Ov. Epi cells 1x10^6 → treat ~~Friday~~ ~~2/8/18~~ Thursday ~~2/12/18~~

2/26/18

~~383~~ NOE

0 ug/mL	383
5 ug/mL	384
20 ug/mL	385
100 ug/mL	386

2/5/18 RNA

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	356	2/5/2018 1:18:50 PM	0.0830	µg/µl	2.074	1.109	1.87	1.30	RNA	40.00
2	357	2/5/2018 1:19:20 PM	0.1000	µg/µl	2.500	1.342	1.86	1.18	RNA	40.00
3	358	2/5/2018 1:19:39 PM	0.0829	µg/µl	2.073	1.118	1.85	1.26	RNA	40.00
4	359	2/5/2018 1:20:00 PM	0.0349	µg/µl	0.873	0.476	1.84	0.39	RNA	40.00
5	360	2/5/2018 1:20:24 PM	0.2387	µg/µl	5.968	2.966	2.01	0.78	RNA	40.00
6	361	2/5/2018 1:20:43 PM	0.3389	µg/µl	8.473	4.194	2.02	1.15	RNA	40.00
7	362	2/5/2018 1:21:04 PM	0.3017	µg/µl	7.542	3.837	1.97	1.47	RNA	40.00
8	363	2/5/2018 1:21:20 PM	0.1118	µg/µl	2.796	1.465	1.91	1.53	RNA	40.00
9	368	2/5/2018 1:21:41 PM	0.2203	µg/µl	5.508	2.880	1.91	1.34	RNA	40.00
10	369	2/5/2018 1:21:57 PM	0.2474	µg/µl	6.185	3.187	1.94	2.03	RNA	40.00
11	370	2/5/2018 1:22:12 PM	0.2217	µg/µl	5.541	2.855	1.94	1.63	RNA	40.00
12	371	2/5/2018 1:22:29 PM	0.1336	µg/µl	3.340	1.726	1.93	1.42	RNA	40.00

#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
1	379	2/16/2018 9:27:37 AM	0.1685	µg/µl	4.212	2.034	2.07	1.01	RNA	40.00
2	380	2/16/2018 9:27:55 AM	0.0658	µg/µl	1.645	0.713	2.31	3.02	RNA	40.00
3	381	2/16/2018 9:28:13 AM	0.0801	µg/µl	2.003	0.891	2.25	0.96	RNA	40.00
4	382	2/16/2018 9:28:30 AM	0.3084	µg/µl	7.711	3.759	2.05	2.24	RNA	40.00
5	383	2/16/2018 9:28:51 AM	0.2921	µg/µl	7.303	3.582	2.04	1.09	RNA	40.00
6	384	2/16/2018 9:29:10 AM	0.1812	µg/µl	4.531	2.179	2.08	2.15	RNA	40.00
7	385	2/16/2018 9:29:29 AM	0.0869	µg/µl	2.172	0.971	2.24	1.31	RNA	40.00
8	386	2/16/2018 9:29:51 AM	0.0116	µg/µl	0.289	-0.017	-16.61	-5.65	RNA	40.00
9	387	2/16/2018 9:30:11 AM	0.0133	µg/µl	0.332	0.013	25.53	-15.74	RNA	40.00
10	395	2/16/2018 9:30:29 AM	0.2169	µg/µl	5.421	2.598	2.09	0.65	RNA	40.00
11	397	2/16/2018 9:30:51 AM	0.1328	µg/µl	3.321	1.575	2.11	2.26	RNA	40.00
12	396	2/16/2018 9:31:08 AM	0.1633	µg/µl	4.084	1.949	2.10	2.47	RNA	40.00

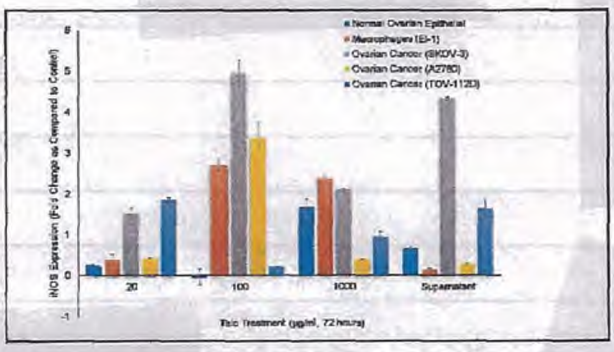
#	Sample ID	Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample Type	Factor
16	364	2/16/2018 9:49:26 AM	0.2401	µg/µl	6.003	2.879	2.09	1.78	RNA	40.00
17	365	2/16/2018 9:49:46 AM	0.2418	µg/µl	6.044	2.939	2.06	1.27	RNA	40.00
18	366	2/16/2018 9:50:01 AM	0.2043	µg/µl	5.106	2.459	2.08	1.88	RNA	40.00
19	367	2/16/2018 9:50:16 AM	0.1712	µg/µl	4.281	2.026	2.11	1.83	RNA	40.00

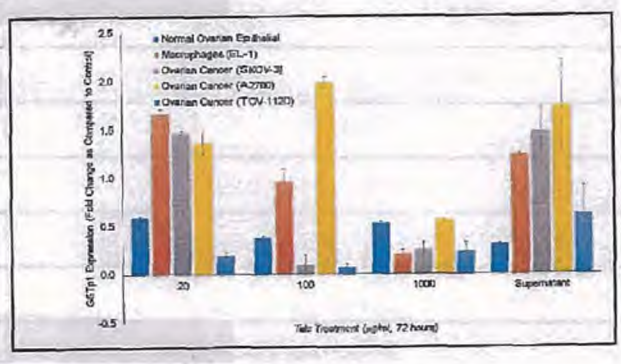
cDNA
- ~~Supers~~ VILO Kit

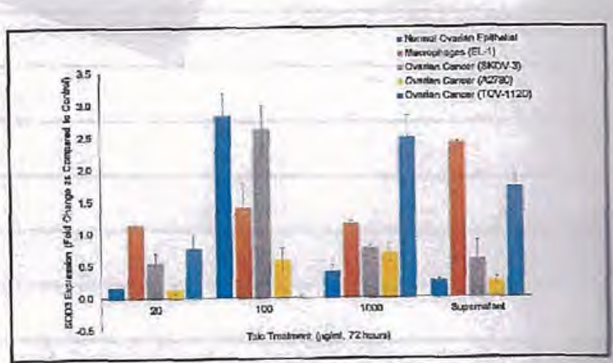
2/16/18

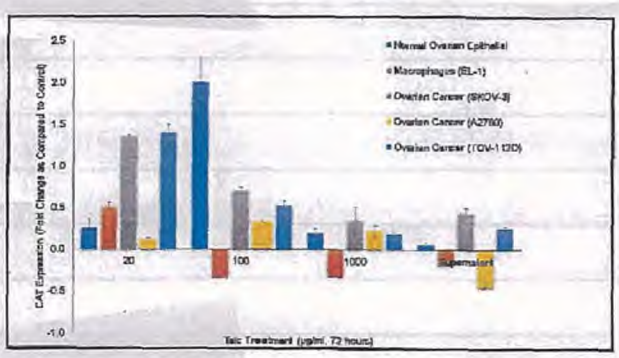
0.5 ug RNA Reaction

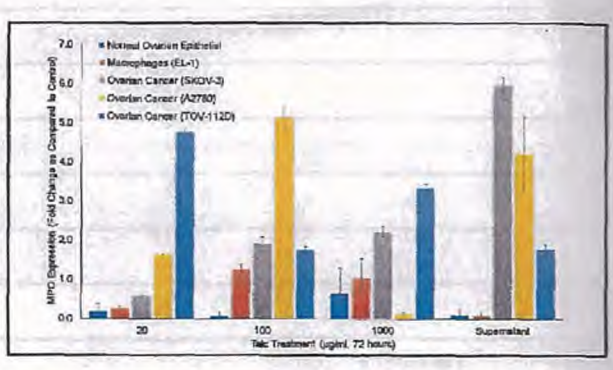
ID	ul RNA	ul water
356	6.0	10.0
357	5.0	11.0
358	6.0	10.0
359	14.3	1.7
360	2.1	13.9
361	1.5	14.5
362	1.7	14.3
363	4.5	11.5
364	2.1	13.9
365	2.1	13.9
366	2.4	13.6
367	2.9	13.1
368	2.3	13.7
369	2.0	14.0
370	2.3	13.7
371	3.7	12.3
379	3.0	13.0
380	7.6	8.4
381	6.2	9.8
382	1.6	14.4
383	1.7	14.3
384	2.8	13.2
385	5.8	10.2
386	16.0	0.0
387	16.0	0.0
395	2.3	13.7
397	3.8	12.2
396	3.1	12.9

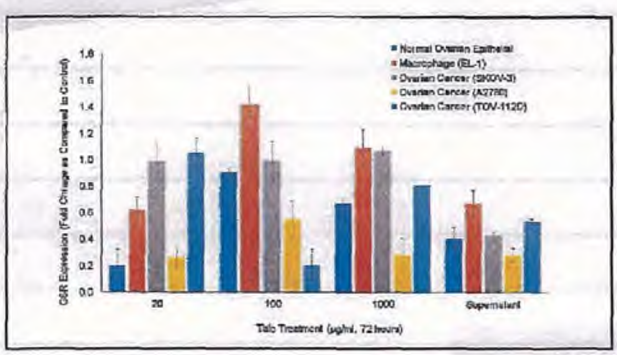

Normal Ovarian Epithelial
Macrophages (EL-1)
Ovarian Cancer (SKOV-3)
Ovarian Cancer (A2780)
Ovarian Cancer (TOV-112D)
iNOS Expression (Fold Change as Compared to Control)
20
100
1000
Supernatant
Talc Treatment (µg/ml, 72 hours)


Normal Ovarian Epithelial
Macrophages (EL-1)
Ovarian Cancer (SKOV-3)
Ovarian Cancer (A2780)
Ovarian Cancer (TOV-112D)
G6Tpl Expression (Fold Change as Compared to Control)
20
100
1000
Supernatant
Talc Treatment (µg/ml, 72 hours)


Normal Ovarian Epithelial
Macrophages (EL-1)
Ovarian Cancer (SKOV-3)
Ovarian Cancer (A2780)
Ovarian Cancer (TOV-112D)
SOD3 Expression (Fold Change as Compared to Control)
20
100
1000
Supernatant
Talc Treatment (µg/ml, 72 hours)


Normal Ovarian Epithelial
Macrophages (EL-1)
Ovarian Cancer (SKOV-3)
Ovarian Cancer (A2780)
Ovarian Cancer (TOV-112D)
CAT Expression (Fold Change as Compared to Control)
20
100
1000
Supernatant
Talc Treatment (µg/ml, 72 hours)


Normal Ovarian Epithelial
Macrophages (EL-1)
Ovarian Cancer (SKOV-3)
Ovarian Cancer (A2780)
Ovarian Cancer (TOV-112D)
MPO Expression (Fold Change as Compared to Control)
20
100
1000
Supernatant
Talc Treatment (µg/ml, 72 hours)


Normal Ovarian Epithelial
Macrophage (EL-1)
Ovarian Cancer (SKOV-3)
Ovarian Cancer (A2780)
Ovarian Cancer (TOV-112D)
GSR Expression (Fold Change as Compared to Control)
20
100
1000
Supernatant
Talc Treatment (µg/ml, 72 hours)

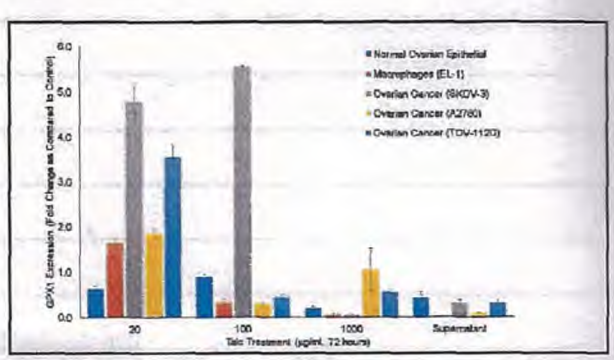

Normal Ovarian Epithelial
Macrophages (EL-1)
Ovarian Cancer (SKOV-3)
Ovarian Cancer (A2780)
Ovarian Cancer (TOV-112D)
GPX1 Expression (Fold Change as Compared to Control)
20
100
1000
Supernatant
Talc Treatment (µg/ml, 72 hours)

Exhibit H

— Cell lines

SKOV-3	ATCC
A2780	Sigma Aldrich, St. Louis, MO)
TOV112D	A kind gift from Guisheng Wu at Wayne State Un
EL-1/macrophages	ATCC,
Normal ovarian epithelial	Cell Biologics, Chicago, IL
FT33	Applied Biological Materials. Richmond, BC, Can

— Fetal bovine serum (FBS, Innovative Research, Novi, MI)
Penicillin/streptomycin (Fisher Scientific)

— Johnson Baby Powder (#30027578 Lot#13717RA)

PCR

Seeded Cells for PCR

1/24/18

- Thawing Cells

	Media
EL-1 (Macrophages)	IMDM (10% FBS, 1% PS, 1mL H-T)
SKOV-3	McCoy's 5A (10% FBS, 1% PS)
TOV112D	Medium 199 : MCDB 105 (1:1) + 10% FBS + 1% P
A2780	RPMI-1640 (10% FBS + 1% PS)
FT33	DMEM (10% FBS, 1% PS)
Normal Ovarian Epithelial	Complete Human Epithelial Cell Medium kit (Cell Biologics)

75 cm² flask + 15mL medium

1/26/18

- Subculture Cells

※ Normal Ovarian Epithelial use trypsin from ScienCell

① Wash with PBS 10mL
② gently remove PBS
③ Pipet trypsin-EDTA 2mL onto the washed cells mono layer
④ 37°C incubator 1 ~ 5 minutes (SKOV-3 longer)
⑤ Check under microscope
⑥ Add fresh medium 8mL to inactivate trypsin. Then Mix
⑦ Take 2mL to a new 100mm dish
⑧ Add 8mL fresh medum to 100mm dish
⑨ Incubate the cells

1/29/18

- Subculture Cells

2mL cells + 8mL medium 100 mm dish

Cells doubled in One day.

SAED000002(color)

2/1/2018

- Subculture cells
- Seeded $1X10^6$ cells 60mm dish + 5ml medium
- Need dose for treatment with talc
  Unt. 5, 20, 100 ug/ml

Sample ID	
356	EL1 Unt
357	EL1 5 ug/ml Talc
358	EL1 20 ug/ml Talc
359	EL1 100 ug/ml Talc
360	SKOV-3 unt
361	SKOV-3 5ug/ml
362	SKOV-3 20ug/ml
363	SKOV-3 100ug/ml
364	TOV112 Unt
365	TOV112 5 ug/ml Talc
366	TOV112 20 ug/ml Talc
367	TOV112 100 ug/ml Talc
368	A2780 Unt
369	A2780 5 ug/ml
370	A2780 20 ug/ml
371	A2780 100 ug/ml
379	FT33 unt
380	FT33 5ug/ml
381	FT33 20 ug/ml
382	FT33 100 ug/ml
383	NOE unt
384	NOE 5 ug/ml Talc
385	NOE 20 ug/ml Talc
386	NOE 100 ug/ml Talc

32

2/2/2018

- treat cell with talc

prepare talc / Johnson Baby Powder (#30027578, lot 15717RA)

100mg talc + 10ml DMSO → mix 10mg/ml = 10^4 ug/ml

- Sterilization under UV light to avoid endotoxin and microbial Contamination
- Powder 100mg suspended in DMSO and passed 5 times through 22-gauge needle and 0.2μl syringe filter

$(X_1)(10^4 \text{ug/ml}) = (5\text{ml})(5\text{ug/ml}) \rightarrow X_1 = 2.5\,\mu l$

$(X_2)(10^4 \text{ug/ml}) = (5\text{ml})(20\text{ug/ml}) \rightarrow X_2 = 10\,\mu l$

$(X_3)(10^4 \text{ug/ml}) = (5\text{ml})(100\text{ug/ml}) \rightarrow X_3 = 50\,\mu l$

2/5/2018

- Collect Cells (See below)
- RNA Extraction RNeasy Mini Kit (Qiagen cat #74106) go to Pg 3
- Detect concentration of RNA by Nanodrop (Thermo Fisher Scientific) go to Pg 35 (Life Technologies)
- cDNA Synthesis via Reverse Transcription – VILO kit go to Pg 35

Cell Collection Protocol

Put on gloves and spray with 70% ethanol
Remove cell culture dish from incubator
Observe cells under microscope.
Move the dishes to your work bench, does not need to be done in the hood.
Collect media and place in labeled 15ml tube for freezing,
Add 10 ml PBS
Using a cell scraper, scrape the bottom of the dish and rotate it to ensure scraping of entire bottom
Using a 10ml pipet, remove the PBS and cell mixture and place into the 15ml conical centrifugation tube that corresponds to the dish, 1ml for RNA, 2ml for DNA,8 ml for protein assay.
Close and centrifuge all tubes, 5 minutes at 1800rpm (slower speed keeps cells from breaking).
Place another paper towel by sink, dump PBS from all tubes into sink and place tubes upside down to drain them. Cells will be collected at the bottom. Place all tubes in Styrofoam holder and place in -80°C freezer.

RNA Extraction

RNeasy Mini Kit (Qiagen cat # 74106)

Important Notes before starting: WORK IN THE HOOD

- β-Mercaptoethanol (β-ME) can be added to Buffer RLT (lysis buffer) before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for **one month** after addition of β-ME.
- Buffer RPE is supplied as a concentrate. Before using for the first time, add ethanol as indicated on the bottle. Be sure to **mark the lid with a X** to show that the working solution has been prepared.

Buffer RW1 and Buffer RLT are hazardous.

- **Buffer RLT+ β-ME should be disposed of in the jar in the hood.**
- **Buffer RWI should be disposed of in the jar in the hood.**

Preparation of the Buffer RLT

- In a labeled 15ml centrifugation tube, add 10µl β-ME for every 1 ml Buffer RLT.

Preparation of your samples

1. Add 350 µl of the Buffer RLT + β-ME solution to each of your sample tubes.
   a. if you have a lot of cells, you will need to add 600 µl of Buffer RLT + β-ME solution to each tube ***also add equal volume of ethanol)
2. Add 350 µl of 70% ethanol to each tube and pipet to mix
3. Transfer the entire sample to its corresponding mini spin column
   a. Close columns and place them into the small centrifuge.
   b. Centrifuge the tubes for 15 seconds at 13,000 rpm
4. Dump the flow through into hazardous waste jar **in the hood**.
5. Add 700µl of the Buffer RW1 to the RNeasy column
   a. Centrifuge 15 seconds at 13,000 rpm
6. Dump the flow through into hazardous waste jar **in the hood**
7. Add 500µl of Buffer RPE onto each RNeasy column
   a. Centrifuge 15 seconds at 13,000 rpm
8. Dump the flow through into waste jar
9. Add 500µl Buffer RPE to each column again
   a. Centrifuge 2 minutes at 13,000 rpm to dry the silica gel membrane
10. Dump the flow through in waste jar, centrifuge for one minute more
11. Remove columns from collection tubes and place in corresponding 1.5ml centrifuge tube
12. Add 50µl of RNase-free water to each column, onto the center of the silica-gel membrane without touching the sides of the column (water dissolves RNA).
    a. Allow to stand for 1 minute
    b. Centrifuge columns for 1 minute at 13,000 rpm, **LID MUST BE ON CENTRIFUGE**
13. Collect flow through from the collection tube and place back into the column on the center of the membrane, allow to stand for 1 minute
    a. Centrifuge columns again for 1 minute at 13,000 rpm, **LID MUST BE ON CENTRIFUGE**
14. Remove and dispose of columns
15. Place your microcentrifuge tubes containing RNA on ice
    a. Detect concentration of RNA
    b. Good quality RNA has a A260/A280 of 2.0

NEED TO MEASURE RNA EACH TIME YOU GO TO MAKE cDNA

cDNA
20 µl

cDNA Synthesis via Reverse Transcription – VILO kit

You will need:
Ice
Thaw, on ice:
RNA
VILO MasterMix
RNase-free water

You must detect the concentration of your RNA. After doing this, you can calculate the volume needed to get for a 1 µg reaction.
i.e. – If your RNA concentration is 0.9 ug/ul then:
(x ul)(0.9 ug/ul) = 1 ug solve for x

For a single reaction, combine the following components in a sterile PCR tube on ice.

	1 µg RNA
Component	**Volume/reaction**
VILO MasterMix	4 µl
Template RNA	Variable up to 1 µg
RNase-free Water	Variable
Total Volume:	20 µl

The total amount in each tube should equal **20 ul**, hence the variable volume of water.

- Add 4 ul VILO MasterMix to each tube, volume of RNA calculated, volume of water calculated, and gently mix.
- Place the tubes in a rack and the rack into a 25°C water bath for 10 minutes.
- Place the rack into a 42°C water bath for 60 minutes.
- Then, place racked tubes into 85°C water bath for 5 minutes to terminate the reaction.
- Place samples on ice for a few minutes.
- Centrifuge cDNA.
- Place into -20°C freezer for storage or continue on to PCR.

RNA Concentration (Nanodrop)

Sample ID		Date and Time	Nucleic Acid Conc.	Unit	A260	A280	260/280	260/230	Sample T
356	EL1 Unt	2/5/2018 1:18:50 PM	0.083	µg/µl	2.074	1.109	1.87	1.3	RNA
357	EL1 5 ug/ml Talc	2/5/2018 1:19:20 PM	0.1	µg/µl	2.5	1.342	1.86	1.18	RNA
358	EL1 20 ug/ml Talc	2/5/2018 1:19:39 PM	0.0829	µg/µl	2.073	1.118	1.85	1.26	RNA
359	EL1 100 ug/ml Talc	2/5/2018 1:20:00 PM	0.0349	µg/µl	0.873	0.476	1.84	0.39	RNA
360	SKOV-3 unt	2/5/2018 1:20:24 PM	0.2387	µg/µl	5.968	2.966	2.01	0.78	RNA
361	SKOV-3 5ug/ml	2/5/2018 1:20:43 PM	0.3389	µg/µl	8.473	4.194	2.02	1.15	RNA
362	SKOV-3 20ug/ml	2/5/2018 1:21:04 PM	0.3017	µg/µl	7.542	3.837	1.97	1.47	RNA
363	SKOV-3 100ug/ml	2/5/2018 1:21:20 PM	0.1118	µg/µl	2.796	1.465	1.91	1.53	RNA
364	TOV112 Unt	2/16/2018 9:49:26 AM	0.2401	µg/µl	6.003	2.879	2.09	1.78	RNA
365	TOV112 5 ug/ml Talc	2/16/2018 9:49:46 AM	0.2418	µg/µl	6.044	2.939	2.06	1.27	RNA
366	TOV112 20 ug/ml Talc	2/16/2018 9:50:01 AM	0.2043	µg/µl	5.106	2.459	2.08	1.88	RNA
367	TOV112 100 ug/ml Tal	2/16/2018 9:50:16 AM	0.1712	µg/µl	4.281	2.026	2.11	1.83	RNA
368	A2780 Unt	2/5/2018 1:21:41 PM	0.2203	µg/µl	5.508	2.88	1.91	1.34	RNA
369	A2780 5 ug/ml	2/5/2018 1:21:57 PM	0.2474	µg/µl	6.185	3.187	1.94	2.03	RNA
370	A2780 20 ug/ml	2/5/2018 1:22:12 PM	0.2217	µg/µl	5.541	2.855	1.94	1.63	RNA
371	A2780 100 ug/ml	2/5/2018 1:22:29 PM	0.1336	µg/µl	3.34	1.726	1.93	1.42	RNA
379	FT33 unt	2/16/2018 9:27:37 AM	0.1685	µg/µl	4.212	2.034	2.07	1.01	RNA
380	FT33 5ug/ml	2/16/2018 9:27:55 AM	0.0658	µg/µl	1.645	0.713	2.31	3.02	RNA
381	FT33 20 ug/ml	2/16/2018 9:28:13 AM	0.0801	µg/µl	2.003	0.891	2.25	0.96	RNA
382	FT33 100 ug/ml	2/16/2018 9:28:30 AM	0.3084	µg/µl	7.711	3.759	2.05	2.24	RNA
383	NOE unt	2/16/2018 9:28:51 AM	0.2921	µg/µl	7.303	3.582	2.04	1.09	RNA
384	NOE 5 ug/ml Talc	2/16/2018 9:29:10 AM	0.1812	µg/µl	4.531	2.179	2.08	2.15	RNA
385	NOE 20 ug/ml Talc	2/16/2018 9:29:29 AM	0.0869	µg/µl	2.172	0.971	2.24	1.31	RNA
386	NOE 100 ug/ml Talc	2/16/2018 9:29:51 AM	0.0816	µg/µl	2.289	1.126	2.03	1.43	RNA

0.5 ug RNA Reaction		
ID	ul RNA	ul water
356	6.0	10.0
357	5.0	11.0
358	6.0	10.0
359	14.3	1.7
360	2.1	13.9
361	1.5	14.5
362	1.7	14.3
363	4.5	11.5
364	2.1	13.9
365	2.1	13.9
366	2.4	13.6
367	2.9	13.1
368	2.3	13.7
369	2.0	14.0
370	2.3	13.7
371	3.7	12.3
379	3.0	13.0
380	7.6	8.4
381	6.2	9.8
382	1.6	14.4
383	1.7	14.3
384	2.8	13.2
385	5.8	10.2
386	6.3	9.7

0.5µg RNA was obtained from each sample following dilution as described by this table.

cDNA (20µL) prepared

SAED000007(color)

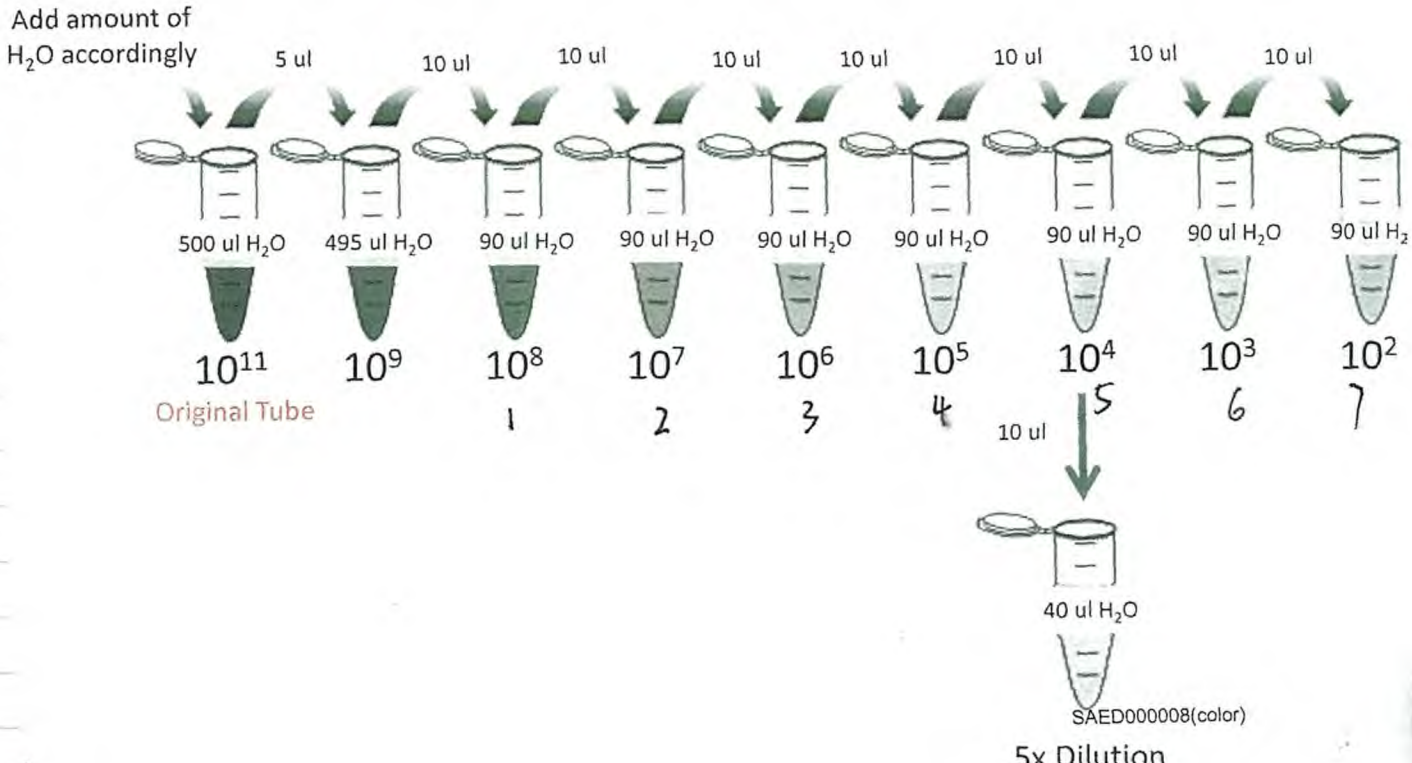
2/19/2018 qRT-PCR for β-actin

β-actin test — standard

- Aliquot Standard
  Standard come desiccated
  Reconstitute with TE buffer.
  Add TE buffer such that the concentration will be 100 μM
  → The volume of TE buffer is on the product sheet
  Mix well
  In a new 1.5ml microtube. add 5ul of standard to each tube
  Put tubes into the concentrator machine for 20 minutes — Lids open
  Then close tubes, and label
  ※ Add 500μl PCR water to get a standard that is 10^{11}

Serial Dilution of Standard

Place samples on ice after mixing



2/19/208

Run β-actin with samples 356 ~ 386

- Do 25μl reaction

Water	9.5μl
Primer Forward	1μl
Primer Reverse	1μl
SYBR Green	12.5μl
Sample (cDNA)	1μl

5μM 20x dilution

(Radiant Green Lo-Rox qPCR Kit #QS105

- Calculating Mastermix for samples

72 samples + 1 blank = 73
73 x 1.17 extra = 85.41

- Mastermix calculation

water = 9.5 x 85.41 = 811.395 ≈ 811.4 μl
primer = 1 x 85.41 = 85.4 μl
SYBR green = 12.5 x 85.41 = 1067.625 = 1067.6 μl

- Mix then take 80.6 of this mix → 1.5ml tube / 1 per sample
  73 x 1.12 extra = 80.6 μl
- Add 3.4μl sample to 1.5ml tube containing mastermix
  3 x 1.12 extra = 3.4 μl

- Mix well. add 25μl → PCR tube.

3 total per sample

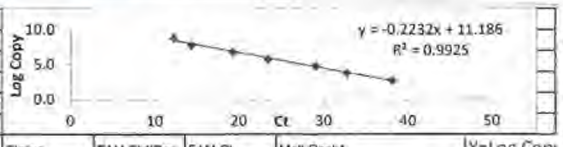
SAED000009(color)

2/19/2018

Run B-actin with Samples 356 – 386

Raw data

n Summary	(Smart Cycler 2.0d)								
n Name:	bactin 1ul 10x								
d Curve:	B-actin Standard RADIANT SYBR								
ated At:	2/19/2018 10:20								
umber of Sites:	72								
esults Table									
te ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Res	FAM Ct	Melt Peak1	Y=Log Cop
8	64-10	8	STD		OK	610000000	12.22	79.62	8.8
9	64-10	7	STD		OK	61000000	14.33	79.66	7.8
10		6	STD		OK	6100000	19.34	79.67	6.8
11	64-10	5	STD		OK	610000	23.46	79.84	5.8
12	64-10	4	STD		OK	61000	29.09	79.92	4.8
13	64-10	3	STD		OK	6100	32.81	80.08	3.8
14	64-10	2	STD		OK	610	36.16	80.41	2.8
1	b-Actin Radiant SYBR 20	356	UNKN		OK	285995.18	25.68	79.46	
2	b-Actin Radiant SYBR 2017		UNKN		OK	273439.209	25.76	79.72	
3	b-Actin Radiant SYBR 2017		UNKN		OK	409589.891	24.98	79.72	
4	b-Actin Radiant SYBR 20	357	UNKN		OK	387206.6	25.09	79.77	
5	b-Actin Radiant SYBR 2017		UNKN		OK	367150.863	25.19	79.65	
6	b-Actin Radiant SYBR 2017		UNKN		OK	378655.448	25.13	79.76	
7	b-Actin Radiant SYBR 20	358	UNKN		OK	230002.825	26.1	79.81	
8	b-Actin Radiant SYBR 2017		UNKN		OK	274451.794	25.76	79.79	
9	b-Actin Radiant SYBR 2017		UNKN		OK	204423.921	26.33	79.5	
A10	b-Actin Radiant SYBR 20	359	UNKN		OK	99410.671	27.73	79.7	
A11	b-Actin Radiant SYBR 2017		UNKN		OK	95098.867	27.82	79.69	
A12	b-Actin Radiant SYBR 2017		UNKN		OK	106962.324	27.59	79.93	
A13	b-Actin Radiant SYBR 20	360	UNKN		OK	82004.156	28.11	79.65	
A14	b-Actin Radiant SYBR 2017		UNKN		OK	76218.669	28.25	79.68	
A15	b-Actin Radiant SYBR 2017		UNKN		OK	80210.088	28.15	79.73	
A16	b-Actin Radiant SYBR 20	361	UNKN		OK	74149.095	28.3	79.69	
B1	b-Actin Radiant SYBR 2017		UNKN		OK	83584.072	28.07	79.9	
B2	b-Actin Radiant SYBR 2017		UNKN		OK	36471.637	29.74	79.85	
B3	b-Actin Radiant SYBR 20	362	UNKN		OK	67751.744	28.48	79.73	
B4	b-Actin Radiant SYBR 2017		UNKN		OK	81987.724	28.11	79.7	
B5	b-Actin Radiant SYBR 2017		UNKN		OK	100552.72	27.71	79.71	
B6	b-Actin Radiant SYBR 20	363	UNKN		OK	77232.773	28.22	79.88	
B7	b-Actin Radiant SYBR 2017		UNKN		OK	73843.031	28.31	79.65	
B8	b-Actin Radiant SYBR 2017		UNKN		OK	74279.769	28.3	79.85	
B9	b-Actin Radiant SYBR 20	364	UNKN		OK	78048.375	28.2	79.75	
B10	b-Actin Radiant SYBR 2017		UNKN		OK	75382.275	28.27	79.89	
B11	b-Actin Radiant SYBR 2017		UNKN		OK	67421.281	28.49	79.75	
B12	b-Actin Radiant SYBR 20	365	UNKN		OK	91845.321	27.89	79.95	
B13	b-Actin Radiant SYBR 2017		UNKN		OK	92266.631	27.88	79.7	
B14	b-Actin Radiant SYBR 2017		UNKN		OK	63374.184	28.61	79.77	
B15	b-Actin Radiant SYBR 20	366	UNKN		OK	41817.434	29.42	79.64	
B16	b-Actin Radiant SYBR 2017		UNKN		OK	49354.598	29.1	79.77	
C1	b-Actin Radiant SYBR 2017		UNKN		OK	65999.285	28.53	79.95	
C2	b-Actin Radiant SYBR 20	367	UNKN		OK	91668.153	27.89	79.92	
C3	b-Actin Radiant SYBR 2017		UNKN		OK	107294.783	27.58	79.68	
C4	b-Actin Radiant SYBR 2017		UNKN		OK	110651.012	27.52	79.89	
C5	b-Actin Radiant SYBR 20	368	UNKN		OK	89094.02	27.95	79.78	
C6	b-Actin Radiant SYBR 2017		UNKN		OK	77572.459	28.22	79.8	
C7	b-Actin Radiant SYBR 2017		UNKN		OK	106760.878	27.59	79.83	
C7	b-Actin Radiant SYBR 20	369	UNKN		OK	136914.317	27.08	79.87	
C8	b-Actin Radiant SYBR 2017		UNKN		OK	22379.944	30.63	79.83	
C9	b-Actin Radiant SYBR 2017		UNKN		OK	198224.635	26.39	79.72	
C11	b-Actin Radiant SYBR 20	370	UNKN		OK	132819.385	27.17	79.76	
C12	b-Actin Radiant SYBR 2017		UNKN		OK	100097.61	27.72	79.74	
C13	b-Actin Radiant SYBR 2017		UNKN		OK	46360.317	29.22	79.63	
C10	b-Actin Radiant SYBR 20	371	UNKN		OK	184842.26	26.52	79.63	
C11	b-Actin Radiant SYBR 2017		UNKN		OK	202714.758	26.35	79.72	
C12	b-Actin Radiant SYBR 2017		UNKN		OK	108192.324	27.57	79.81	
A1	b-Actin Radiant SYBR 20	379	UNKN		OK	307932.328	25.53	79.52	
A2	b-Actin Radiant SYBR 2017		UNKN		OK	377133.607	25.14	79.75	
A3	b-Actin Radiant SYBR 2017		UNKN		OK	542309.187	24.43	79.71	
A4	b-Actin Radiant SYBR 20	380	UNKN		OK	315038.876	25.49	79.88	
A5	b-Actin Radiant SYBR 2017		UNKN		OK	251730.241	25.92	79.59	
A6	b-Actin Radiant SYBR 2017		UNKN		OK	310158.171	25.52	79.58	
A7	b-Actin Radiant SYBR 20	381	UNKN		OK	328994.514	25.4	79.76	
A8	b-Actin Radiant SYBR 2017		UNKN		OK	298610.861	25.6	79.69	
A9	b-Actin Radiant SYBR 2017		UNKN		OK	271028.804	25.78	79.38	
A10	b-Actin Radiant SYBR 20	382	UNKN		OK	202182.58	26.35	79.57	
A11	b-Actin Radiant SYBR 2017		UNKN		OK	176291.01	26.62	79.73	
A12	b-Actin Radiant SYBR 2017		UNKN		OK	204290.69	26.33	79.76	
A13	b-Actin Radiant SYBR 20	383	UNKN		OK	188116.97	26.49	79.53	
A14	b-Actin Radiant SYBR 2017		UNKN		OK	176951.812	26.61	79.61	
A15	b-Actin Radiant SYBR 2017		UNKN		OK	185011.186	26.52	79.68	
A16	b-Actin Radiant SYBR 20	384	UNKN		OK	128937.209	27.23	79.69	
B1	b-Actin Radiant SYBR 2017		UNKN		OK	128406.079	27.23	79.78	
B2	b-Actin Radiant SYBR 2017		UNKN		OK	74621.79	28.29	79.68	
B3	b-Actin Radiant SYBR 20	385	UNKN		OK	115595.389	27.44	79.61	
B4	b-Actin Radiant SYBR 2017		UNKN		OK	160609.197	26.8	79.74	
B5	b-Actin Radiant SYBR 2017		UNKN		OK	172272.153	26.66	79.8	
B6	b-Actin Radiant SYBR 20	386	UNKN		OK	96398.19	27.79	79.9	
B7	b-Actin Radiant SYBR 2017		UNKN		OK	78347.732	28.2	79.71	
B8	b-Actin Radiant SYBR 2017		UNKN		OK	77149.198	28.23	79.78	



SAED000010(CONF)

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product /Amplicon Length	Start Position
NM_001101	B-actin	ATGACTTAGTTGCGTTACACCCTTTCTTGACAAAACCTAACTTGCGCAGAAAACAAGATGAGATTGGCATGGCTTTATT	ATGACTTAGTTGCGTTAC	AATAAAGCCATGCCAATCT	79	79	1220

Calculation data

Initial time (s) at 95 C	Melt time at 95 C	Annaal time (s) and Temp	extension time (s) and temp
60	15	10, 58	30, 72

ene of interest	B-actin		
		Unit:	Formula
Dalton = 1.66E-24 grams	1.66E-24	g	
ass of base pair	615	Da	
g. Mass/base	305.25	Da	
ngth of entire gene	79	bases	
ass in Daltons	2.41E-04	Da	= number bases x avg. mass/base
ass in grams	4.00E-20	g	= mass in Da x mass of a Da in grams
ass in ug	4.00E-14	ug	=above / 10E-6
ass in ng	4.00E-11	ng/rxn	= above x 10E3

/19/2018 10:20

D	Sample	Copy #	ul cDNA used	copies/ul cDNA	ug RNA used	ul cDNA made	ug RNA/ul cDNA	copies/ng RNA	Dilution Factor	Copies/ug RNA x Df	pg/ug RNA	Avg	Normalized Avg
356	EL1 Unt	285995.18	1	285995	0.5	20	0.025	1.14E+07	10	1.14E+08	4.58	4.48	1.03
		273439.21	1	273439	0.5	20	0.025	1.09E+07	10	1.09E+08	4.38		
		409589.89	1	409590	0.5	20	0.025	1.64E+07	10	1.64E+08	6.56		
357	EL1 5 ug/ml Talc	287205.6	1	287207	0.5	20	0.025	1.15E+07	10	1.15E+08	4.60	4.45	1.02
		267150.86	1	267151	0.5	20	0.025	1.07E+07	10	1.07E+08	4.29		
		278655.45	1	278655	0.5	20	0.025	1.11E+07	10	1.11E+08	4.46		
358	EL1 20 ug/ml Talc	230002.83	1	230003	0.5	20	0.025	9.20E+06	10	9.20E+07	3.68	3.48	0.80
		274451.79	1	274452	0.5	20	0.025	1.10E+07	10	1.10E+08	4.39		
		204423.92	1	204424	0.5	20	0.025	8.18E+06	10	8.18E+07	3.27		
359	EL1 100 ug/ml Talc	294104.67	1	294105	0.5	20	0.025	1.18E+07	10	1.18E+08	4.71	4.36	1.00
		250982.87	1	250983	0.5	20	0.025	1.00E+07	10	1.00E+08	4.02		
		246925.32	1	246925	0.5	20	0.025	9.88E+06	10	9.88E+07	3.95		
360	SKOV-3 Unt	82004.156	1	82004.2	0.5	20	0.025	3.28E+06	10	3.28E+07	1.31	1.27	1.06
		76218.659	1	76218.7	0.5	20	0.025	3.05E+06	10	3.05E+07	1.22		
		80210.088	1	80210.1	0.5	20	0.025	3.21E+06	10	3.21E+07	1.28		
361	SKOV-3 5 ug/ml	74149.095	1	74149.1	0.5	20	0.025	2.97E+06	10	2.97E+07	1.19	1.26	1.05
		83584.072	1	83584.1	0.5	20	0.025	3.34E+06	10	3.34E+07	1.34		
		35471.637	1	35471.6	0.5	20	0.025	1.42E+06	10	1.42E+07	0.57		
362	SKOV-3 20 ug/ml	67751.744	1	67751.7	0.5	20	0.025	2.71E+06	10	2.71E+07	1.08	1.33	1.11
		81687.724	1	81687.7	0.5	20	0.025	3.27E+06	10	3.27E+07	1.31		
		100652.72	1	100653	0.5	20	0.025	4.03E+06	10	4.03E+07	1.61		
363	SKOV-3 100 ug/ml	77232.773	1	77232.8	0.5	20	0.025	3.09E+06	10	3.09E+07	1.24	1.20	1.00
		73843.031	1	73843	0.5	20	0.025	2.95E+06	10	2.95E+07	1.18		
		74279.769	1	74279.8	0.5	20	0.025	2.97E+06	10	2.97E+07	1.19		
364	TOV112 Unt	78048.375	1	78048.4	0.5	20	0.025	3.12E+06	10	3.12E+07	1.25	1.24	1.69
		75382.275	1	75382.3	0.5	20	0.025	3.02E+06	10	3.02E+07	1.21		
		67421.281	1	67421.3	0.5	20	0.025	2.70E+06	10	2.70E+07	1.08		
365	TOV112 5 ug/ml Tal	91845.321	1	91845.3	0.5	20	0.025	3.67E+06	10	3.67E+07	1.47	1.47	2.02
		92266.631	1	92266.6	0.5	20	0.025	3.69E+06	10	3.69E+07	1.48		
		63374.184	1	63374.2	0.5	20	0.025	2.53E+06	10	2.53E+07	1.01		
366	TOV112 20 ug/ml T	41817.434	1	41817.4	0.5	20	0.025	1.67E+06	10	1.67E+07	0.67	0.73	1.00
		49354.598	1	49354.6	0.5	20	0.025	1.97E+06	10	1.97E+07	0.79		
		65999.285	1	65999.3	0.5	20	0.025	2.64E+06	10	2.64E+07	1.06		
367	TOV112 100 ug/ml	91668.153	1	91668.2	0.5	20	0.025	3.67E+06	10	3.67E+07	1.47	1.74	2.39
		107294.78	1	107295	0.5	20	0.025	4.29E+06	10	4.29E+07	1.72		
		110651.01	1	110651	0.5	20	0.025	4.43E+06	10	4.43E+07	1.77		
368	A2780 Unt	89094.02	1	89094	0.5	20	0.025	3.56E+06	10	3.56E+07	1.43	1.33	1.00
		77572.459	1	77572.5	0.5	20	0.025	3.10E+06	10	3.10E+07	1.24		
		106760.88	1	106761	0.5	20	0.025	4.27E+06	10	4.27E+07	1.71		
369	A2780 5 ug/ml	138914.32	1	138914	0.5	20	0.025	5.56E+06	10	5.56E+07	2.22	2.70	2.02
		22379.944	1	22379.9	0.5	20	0.025	8.95E+05	10	8.95E+06	0.36		
		198224.64	1	198225	0.5	20	0.025	7.93E+06	10	7.93E+07	3.17		
370	A2780 20 ug/ml	132819.39	1	132819	0.5	20	0.025	5.31E+06	10	5.31E+07	2.13	1.86	1.40
		100097.61	1	100098	0.5	20	0.025	4.00E+06	10	4.00E+07	1.60		
		46360.317	1	46360.3	0.5	20	0.025	1.85E+06	10	1.85E+07	0.74		
371	A2780 100 ug/ml	184842.26	1	184842	0.5	20	0.025	7.39E+06	10	7.39E+07	2.96	3.10	2.33
		202714.76	1	202715	0.5	20	0.025	8.11E+06	10	8.11E+07	3.25		
		108192.32	1	108192	0.5	20	0.025	4.33E+06	10	4.33E+07	1.73		
372	FT33 Unt	307932.33	1	307933	0.5	20	0.025	1.23E+07	10	1.23E+08	4.93	5.48	1.35
		377133.61	1	377134	0.5	20	0.025	1.51E+07	10	1.51E+08	6.04		
		542309.19	1	542309	0.5	20	0.025	2.17E+07	10	2.17E+08	8.68		
380	FT33 5ug/ml	315038.88	1	315039	0.5	20	0.025	1.26E+07	10	1.26E+08	5.04	4.54	1.04
		251730.24	1	251730	0.5	20	0.025	1.01E+07	10	1.01E+08	4.03		
		310158.17	1	310158	0.5	20	0.025	1.24E+07	10	1.24E+08	4.97		
381	FT33 20ug/ml	328994.51	1	328995	0.5	20	0.025	1.32E+07	10	1.32E+08	5.27	4.54	1.04
		296610.66	1	296611	0.5	20	0.025	1.19E+07	10	1.19E+08	4.75		
		271028.8	1	271029	0.5	20	0.025	1.08E+07	10	1.08E+08	4.34		
382	FT33 100 ug/ml	202182.58	1	202183	0.5	20	0.025	8.09E+06	10	8.09E+07	3.24	3.25	2.71
		176291.01	1	176291	0.5	20	0.025	7.05E+06	10	7.05E+07	2.82		
		204290.69	1	204291	0.5	20	0.025	8.17E+06	10	8.17E+07	3.27		
383	NOE unt	188116.97	1	188117	0.5	20	0.025	7.52E+06	10	7.52E+07	3.01	2.94	2.44
		176951.81	1	176952	0.5	20	0.025	7.08E+06	10	7.08E+07	2.83		
		185011.19	1	185011	0.5	20	0.025	7.40E+06	10	7.40E+07	2.96		
384	NOE 5ug/ml	128937.21	1	128937	0.5	20	0.025	5.16E+06	10	5.16E+07	2.06	2.06	1.71
		128406.08	1	128406	0.5	20	0.025	5.14E+06	10	5.14E+07	2.06		
		74621.79	1	74621.8	0.5	20	0.025	2.98E+06	10	2.98E+07	1.19		
385	NOE 20ug/ml	115595.39	1	115595	0.5	20	0.025	4.62E+06	10	4.62E+07	1.85	2.67	2.00
		160609.2	1	160609	0.5	20	0.025	6.42E+06	10	6.42E+07	2.57		
		172272.15	1	172272	0.5	20	0.025	6.89E+06	10	6.89E+07	2.76		
386	NOE 100 ug/ml	96396.19	1	96396.2	0.1856	20	0.00928	1.04E+07	10	1.04E+08	4.16	3.35	2.51
		78347.732	1	78347.7	0.1856	20	0.00928	8.44E+06	10	8.44E+07	3.38		
		77149.198	1	77149.2	0.1856	20	0.00928	8.31E+06	10	8.31E+07	3.33		

SAED00011(color)

2/19/2018

Run qPCR CAT with samples 356 ~ 386

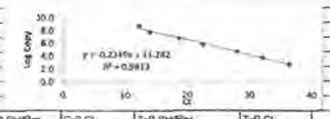
Primer info

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product /Amplicon Length	Start Position
NM_001852 Ordered Feb 2015	CAT	GGTTGAACAGATAGCCTTCGACCCAAGCAACATGCCAC CTGGCATTGAGGCCAGTCCTGACAAAATGCTTCAGGGC CGCCTTTTTGCCTATCCTGACACTCACCG	GGTTGAACAGATAGCCTTC	CGGTGAGTGTCAGGATAG	105	105	1073

Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp	extension time (s) and temp
60	15	10, 60	30,72

Raw data

Run Summary (Smart Cycler 2.0d)
Run Name: CAT 356-370 3ul 10x
Std Curve: CAT Standard RADIANT SYBR
Started At: 2/19/2018 14:30
Number of S: 53



Results Table

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Rc	FAM Ct	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct		Cy5 Ct	Melt Peak1	Y=Log Copy
C15	67-10	8	STD		OK	606000000	12.2	0	16.91	0	0		0	85.45	8.8
C16	67-10	7	STD		OK	60600000	13.94	0	0	0	0		0	85.38	7.8
D1	67-10	6	STD		OK	6060000.5	16.73	0	23.83	0	0		0	85.46	6.8
D2	67-10	5	STD		OK	606000	22.55	0	29.61	0	0		0	85.43	5.8
D3	67-10	4	STD		OK	60600	27.97	0	32.92	0	0		0	85.47	4.8
D4	67-10	3	STD		OK	6060	32.1	0	37.43	0	0		0	85.61	3.8
D5	67-10	2	STD		OK	606	36.4	0	0	0	0		0	85.74	2.8
A2	CAT - RADIANT SYBR 2017	356	UNKN		OK	17855.978	29.94	17678.93714	0	ND	0		0	85.43	
A3	CAT - RADIANT SYBR 2017		UNKN		OK	16139.221	30.13	15950.97106	0	ND	0		0	85.56	
A4	CAT - RADIANT SYBR 2017		UNKN		OK	4915.959	32.32	4874.36504	0	ND	0		0	85.49	
A5	CAT - RADIANT SYBR 2017	357	UNKN		OK	12459.444	30.59	12400.50223	0	ND	0		0	85.36	
A6	CAT - RADIANT SYBR 2017		UNKN		OK	10272.547	30.96	10177.80459	0	ND	0		0	85.43	
A7	CAT - RADIANT SYBR 2017		UNKN		OK	14937.016	30.27	14786.76389	0	ND	0		0	85.33	
A8	CAT - RADIANT SYBR 2017	358	UNKN		OK	80205.3	31.41	7985.839205	0	ND	0		0	85.49	
A9	CAT - RADIANT SYBR 2017		UNKN		OK	8563.013	31.29	8521.143499	0	ND	0		0	85.18	
A10	CAT - RADIANT SYBR 2017		UNKN		OK	7784.922	31.46	7748.542888	0	ND	0		0	85.15	
A11	CAT - RADIANT SYBR 2017	359	UNKN		OK	5724.982	32.03	5698.992893	0	ND	0		0	85.3	
A12	CAT - RADIANT SYBR 2017		UNKN		OK	5383.508	32.15	5356.12657	0	ND	0		0	85.56	
A13	CAT - RADIANT SYBR 2017		UNKN		OK	4531.314	32.47	4494.20671	0	ND	0		0	85.51	
A14	CAT - RADIANT SYBR 2017	360	UNKN		OK	1178.489	30.79	11125.10317	0	ND	0		0	85.44	
A15	CAT - RADIANT SYBR 2017		UNKN		OK	1030.117	30.98	10081.30027	0	ND	0		0	85.47	
A16	CAT - RADIANT SYBR 2017		UNKN		OK	1112.669	30.97	10103.75917	0	ND	0		0	85.64	
B1	CAT - RADIANT SYBR 2017	361	UNKN		OK	1720.575	35.67	713.3068093	0	ND	0		0	85.69	
B2	CAT - RADIANT SYBR 2017		UNKN		OK	1536.288	36.42	529.6730491	0	ND	0		0	85.7	
B3	CAT - RADIANT SYBR 2017		UNKN		OK	1454.544	36.72	152.502786	0	ND	0		0	85.65	
B4	CAT - RADIANT SYBR 2017	362	UNKN		OK	988.97	35.29	976.4970234	0	ND	0		0	85.51	
B5	CAT - RADIANT SYBR 2017		UNKN		OK	892.134	36.24	583.8830707	0	ND	0		0	85.65	
B6	CAT - RADIANT SYBR 2017		UNKN		OK	743.008	37.24	339.8015296	0	ND	0		0	85.71	
B7	CAT - RADIANT SYBR 2017	363	UNKN		OK	930.122	35.40	920.0467999	0	ND	0		0	85.51	
B8	CAT - RADIANT SYBR 2017		UNKN		OK	891.701	35.48	881.0627594	0	ND	0		0	85.47	
B9	CAT - RADIANT SYBR 2017		UNKN		OK	917.542	35.60	162.736303	0	ND	0		0	85.56	
B10	CAT - RADIANT SYBR 2017	364	UNKN		OK	9774.808	31.04	9727.581426	0	ND	0		0	85.78	
B11	CAT - RADIANT SYBR 2017		UNKN		OK	8772.54	31.24	8729.736841	0	ND	0		0	85.51	
B12	CAT - RADIANT SYBR 2017		UNKN		OK	7079.596	31.64	7044.40926	0	ND	0		0	85.82	
B13	CAT - RADIANT SYBR 2017	365	UNKN		OK	6450.657	31.81	5418.341978	0	ND	0		0	85.52	
B14	CAT - RADIANT SYBR 2017		UNKN		OK	6345.112	31.84	6313.281374	0	ND	0		0	85.73	
B15	CAT - RADIANT SYBR 2017		UNKN		OK	6548.981	31.78	6516.215381	0	ND	0		0	83.96	
B16	CAT - RADIANT SYBR 2017	366	UNKN		OK	4356.817	32.54	4334.257044	0	ND	0		0	85.61	
C1	CAT - RADIANT SYBR 2017		UNKN		OK	4125.06	32.64	4103.614077	0	ND	0		0	85.67	
C2	CAT - RADIANT SYBR 2017		UNKN		OK	4205.28	32.60	4183.45132	0	ND	0		0	85.83	
C4	CAT - RADIANT SYBR 2017	367	UNKN		OK	470.667	30.59	12400.50223	0	ND	0		0	85.53	
C5	CAT - RADIANT SYBR 2017		UNKN		OK	269.742	30.96	10177.80459	0	ND	0		0	85.57	
C6	CAT - RADIANT SYBR 2017		UNKN		OK	832.75	30.27	14786.76386	0	ND	0		0	85.72	
C7	CAT - RADIANT SYBR 2017	368	UNKN		OK	13187.089	30.49	13125.00211	0	ND	0		0	85.75	
C8	CAT - RADIANT SYBR 2017		UNKN		OK	11210.87	30.79	11157.33328	0	ND	0		0	85.58	
C9	CAT - RADIANT SYBR 2017		UNKN		OK	12105.843	30.65	12048.42934	0	ND	0		0	85.78	
C10	CAT - RADIANT SYBR 2017	369	UNKN		OK	7785.962	31.46	7747.586091	0	ND	0		0	85.66	
C11	CAT - RADIANT SYBR 2017		UNKN		OK	9185.252	31.19	9140.612872	0	ND	0		0	85.56	
C12	CAT - RADIANT SYBR 2017		UNKN		OK	8973.241	31.20	8929.543543	0	ND	0		0	85.59	
C13	CAT - RADIANT SYBR 2017	370	UNKN		OK	6524.891	31.79	6492.235722	0	ND	0		0	85.61	
C14	CAT - RADIANT SYBR 2017		UNKN		OK	5247.982	32.19	5221.233205	0	ND	0		0	85.66	
C15	CAT - RADIANT SYBR 2017		UNKN		OK	5986.997	31.95	5956.915555	0	ND	0		0		

2/19/2018

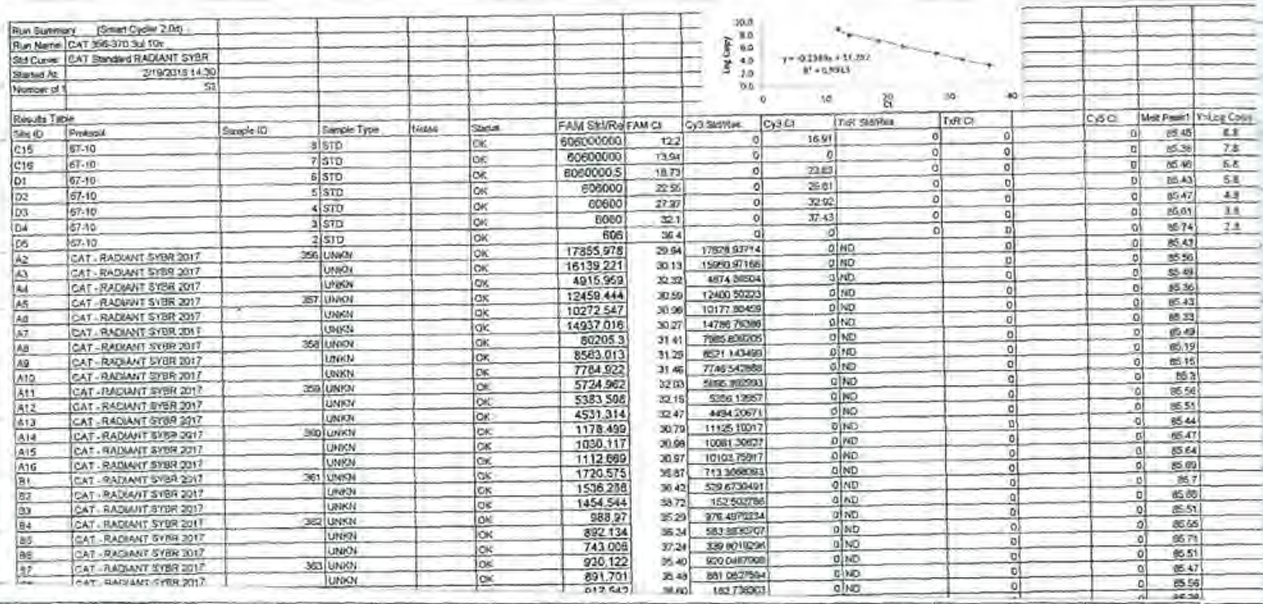
Run qPCR CAT with samples 356 ~ 386

Primer info

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product /Amplicon Length	Start Position
NM_001852 Ordered Feb 2015	CAT	GGTTGAACAGATAGCCTTCGACCCAAGCAACATGCCAC CTGGCATTGAGGCCAGTCCTGACAAAATGCTTCAGGGC CGCCTTTTTGCCTATCCTGACACTCACCG	GGTTGAACAGATAGCCTTC	CGGTGAGTGTCAGGATAG	105	105	1073

Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp	extension time (s) and temp
60	15	10, 60	30,72

Raw data



Run Summary (Smart Cycler 2.0d)	
Run Name	CAT 356-370 3ul 10x
Std Curve	CAT Standard RADIANT SYBR
Started At	2/19/2018 14:30
Number of	53

Results Table

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Rxn	FAM Ct	Cy3 Std/Rxn	Cy3 Ct	TxR Std/Rxn	TxR Ct		Cy5 Ct	Melt Peak1	Y-Log Conc
C15	67-10	8	STD		OK	606000000	12.2	0	16.91	0	0		0	85.45	8.8
C16	67-10	7	STD		OK	60600000	13.94	0	0	0	0		0	85.36	7.8
D1	67-10	6	STD		OK	6060000.5	18.73	0	23.83	0	0		0	85.46	6.8
D2	67-10	5	STD		OK	606000	22.55	0	26.61	0	0		0	85.43	5.8
D3	67-10	4	STD		OK	60600	27.97	0	32.92	0	0		0	85.47	4.8
D4	67-10	3	STD		OK	6060	32.1	0	37.43	0	0		0	85.61	3.8
D5	67-10	2	STD		OK	606	36.4	0	0	0	0		0	85.74	2.8
A2	CAT - RADIANT SYBR 2017	356	UNKN		OK	17855.978	29.94	17826.93714	0	ND			0	85.43	
A3	CAT - RADIANT SYBR 2017		UNKN		OK	16139.221	30.13	15990.97166	0	ND			0	85.56	
A4	CAT - RADIANT SYBR 2017		UNKN		OK	4915.959	32.32	4874.98504	0	ND			0	85.49	
A5	CAT - RADIANT SYBR 2017	357	UNKN		OK	12459.444	30.59	12400.50223	0	ND			0	85.36	
A6	CAT - RADIANT SYBR 2017		UNKN		OK	10272.547	30.96	10177.80409	0	ND			0	85.43	
A7	CAT - RADIANT SYBR 2017		UNKN		OK	14937.016	30.27	14786.78288	0	ND			0	85.33	
A8	CAT - RADIANT SYBR 2017	358	UNKN		OK	80205.3	31.41	7985.806205	0	ND			0	85.49	
A9	CAT - RADIANT SYBR 2017		UNKN		OK	8583.013	31.29	8521.143499	0	ND			0	85.19	
A10	CAT - RADIANT SYBR 2017		UNKN		OK	7784.922	31.46	7746.542868	0	ND			0	85.15	
A11	CAT - RADIANT SYBR 2017	359	UNKN		OK	5734.962	32.03	5695.860093	0	ND			0	85.3	
A12	CAT - RADIANT SYBR 2017		UNKN		OK	5383.596	32.15	5356.13957	0	ND			0	85.56	
A13	CAT - RADIANT SYBR 2017		UNKN		OK	4531.314	32.47	4494.20671	0	ND			0	85.51	
A14	CAT - RADIANT SYBR 2017	360	UNKN		OK	1178.499	30.79	11925.10017	0	ND			0	85.44	
A15	CAT - RADIANT SYBR 2017		UNKN		OK	1030.117	30.98	10081.30627	0	ND			0	85.47	
A16	CAT - RADIANT SYBR 2017		UNKN		OK	1112.669	30.97	10103.75917	0	ND			0	85.64	
B1	CAT - RADIANT SYBR 2017	361	UNKN		OK	1720.575	30.87	713.3696093	0	ND			0	85.69	
B2	CAT - RADIANT SYBR 2017		UNKN		OK	1536.238	30.42	529.6730491	0	ND			0	86.7	
B3	CAT - RADIANT SYBR 2017		UNKN		OK	1454.544	30.72	152.502786	0	ND			0	85.86	
B4	CAT - RADIANT SYBR 2017	362	UNKN		OK	988.97	35.29	976.4970234	0	ND			0	85.51	
B5	CAT - RADIANT SYBR 2017		UNKN		OK	892.134	35.34	583.8830707	0	ND			0	86.55	
B6	CAT - RADIANT SYBR 2017		UNKN		OK	743.006	37.24	339.8010294	0	ND			0	85.71	
B7	CAT - RADIANT SYBR 2017	363	UNKN		OK	930.122	35.40	920.0487008	0	ND			0	85.51	
B8	CAT - RADIANT SYBR 2017		UNKN		OK	891.701	35.49	881.0827594	0	ND			0	85.47	
						612.643	35.60	182.736003	0	ND			0	85.56	

Run Summary (Smart Cycler 2.0d)	
Run Name	CAT 3 ul at 10x 371-397
Std Curve	CAT Standard RADIANT SYBR
Started At	2/19/2018 17:17
Number of	45

Results Table

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/Rxn	30.52	Cy3 Std/Rxn	Cy3 Ct	TxR Std/Rxn	TxR Ct		Cy5 Ct	Melt Peak1
C15	67-10	8	STD		OK	606000000	32.63	0	16.91	0	0		0	85.45
C16	67-10	7	STD		OK	60600000	32.56	0	0	0	0		0	85.38
D1	67-10	6	STD		OK	6060000.5	36.35	0	23.83	0	0		0	85.46
D2	67-10	5	STD		OK	606000	35.99	0	29.61	0	0		0	85.43
D3	67-10	4	STD		OK	60600	38.00	0	32.92	0	0		0	85.47
D4	67-10	3	STD		OK	6060	39.24	0	37.43	0	0		0	85.61
D5	67-10	2	STD		OK	606	39.22	0	0	0	0		0	85.74
A1	CAT - RADIANT SYBR 2017	371	UNKN		OK	1532.565	34.47	1523.954788	0	ND			0	85.27
A2	CAT - RADIANT SYBR 2017		UNKN		OK	1236.764	34.86	1229.703382	0	ND			0	85.52
A3	CAT - RADIANT SYBR 2017		UNKN		OK	1250.345	34.84	1243.212629	0	ND			0	85.7
A4	CAT - RADIANT SYBR 2017	379	UNKN		OK	13751.972	31.09	6488.709467	0	ND			0	85.53
A5	CAT - RADIANT SYBR 2017		UNKN		OK	14752.394	31.34	8281.795838	0	ND			0	85.48
A6	CAT - RADIANT SYBR 2017		UNKN		OK	15528.954	31.49	7841.631079	0	ND			0	85.32
A7	CAT - RADIANT SYBR 2017	380	UNKN		OK	12011.51	31.84	8312.883701	0	ND			0	85.46
A8	CAT - RADIANT SYBR 2017		UNKN		OK	15456.092	31.86	6257.764643	0	ND			0	85.32
A9	CAT - RADIANT SYBR 2017		UNKN		OK	14021.656	31.78	8523.18253	0	ND			0	85.15
A10	CAT - RADIANT SYBR 2017	381	UNKN		OK	9278.117	32.52	4375.630518	0	ND			0	85.52
A11	CAT - RADIANT SYBR 2017		UNKN		OK	8741.24	32.63	4123.440981	0	ND			0	85.4
A12	CAT - RADIANT SYBR 2017		UNKN		OK	5417.343	32.56	4277.101571	0	ND			0	85.3
A13	CAT - RADIANT SYBR 2017	382	UNKN		OK	11019.777	36.35	550.986046	0	ND			0	85.59
A14	CAT - RADIANT SYBR 2017		UNKN		OK	10563.774	35.99	668.9056823	0	ND			0	85.43
A15	CAT - RADIANT SYBR 2017		UNKN		OK	9467.413	36.00	664.9291676	0	ND			0	85.48
A16	CAT - RADIANT SYBR 2017	383	UNKN		OK	9763.023	36.24	15035.75313	0	ND			0	85.4
B1	CAT - RADIANT SYBR 2017		UNKN		OK	8932.902	36.22	15218.38365	0	ND			0	85.7
B2	CAT - RADIANT SYBR 2017		UNKN		OK	7723.593	36.20	15315.80267	0	ND			0	85.56
B3	CAT - RADIANT SYBR 2017	384	UNKN		OK	4509.33	34.09	1888.845906	0	ND			0	85.66
B4	CAT - RADIANT SYBR 2017		UNKN		OK	4792.009	34.16	1779.286131	0	ND			0	85.14
B5	CAT - RADIANT SYBR 2017		UNKN		OK	3980.332	34.06	1876.863202	0	ND			0	85.61
B6	CAT - RADIANT SYBR 2017	385	UNKN		OK	14691.73	35.48	883.7826164	0	ND			0	85.55
B7	CAT - RADIANT SYBR 2017		UNKN		OK	13686.861	35.91	886.9833264	0	ND			0	85.38
B8	CAT - RADIANT SYBR 2017		UNKN		OK	12783.956	35.64	907.2107853	0	ND			0	85.7
B9	CAT - RADIANT SYBR 2017	386	UNKN		OK	11571.379	35.98	673.8852469	0	ND			0	85.36
B10	CAT - RADIANT SYBR 2017		UNKN		OK	11590.693	36.20	895.9832513	0	ND			0	85.68
B11	CAT - RADIANT SYBR 2017		UNKN		OK	12700.554	36.13	819.2435347	0	ND			0	85.48

SAED000013(color)

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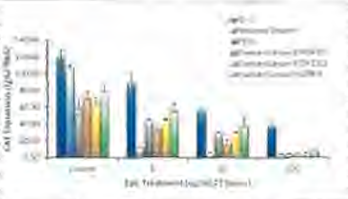
Calculation data

Gene of Interest	CAT		
		Unit	Formula
1 Dalton = 1.66E-24 grams	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire gene	105	bases	
Mass in Daltons	3.21E+04	Da	= num/bers bases x avg. mass/base
Mass in grams	5.32E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	5.32E-14	ug	=above / 10E-6
Mass in ng	5.32E-11	ng/copy	=above x 10E3

67-10

ID	Sample	Copy #	ul cDNA used	copies/ul cDNA	ug RNA used	ul cDNA made	ug RNA/ul cDNA	copies/ug RNA	Dilution Factor	Copies/ug RNA x DF	fg/ug RNA	Normalized	Avg	SD		
356	Unt	17760.287836	3	5920.1	0.5	20	0.025	2.37E+05	10	2.37E+06	125.99	122.76	119.84	8.704027	Control	
	EL1 Unt 72 hr	16025.072389	3	5341.69	0.5	20	0.025	2.14E+05	10	2.14E+06	113.68					
		4889.480122	3	1629.16	0.5	20	0.025	6.53E+04	10	6.53E+05	34.76					
357	5 ug/ml	12459.444000	3	4153.15	0.5	20	0.025	1.66E+05	10	1.66E+06	88.39	86.76	88.97	11.19853		5
	EL1 5 ug/ml Talc	10227.041070	3	3409.01	0.5	20	0.025	1.36E+05	10	1.36E+06	72.55					
		14855.935323	3	4951.98	0.5	20	0.025	1.98E+05	10	1.98E+06	105.39					
358	20 ug/ml	8026.300000	3	2675.1	0.5	20	0.025	1.07E+05	10	1.07E+06	56.93	71.43	56.08	2.626264		20
	EL1 20 ug/ml Talc	8563.013000	3	2854.34	0.5	20	0.025	1.14E+05	10	1.14E+06	60.75					
		7784.922000	3	2594.97	0.5	20	0.025	1.04E+05	10	1.04E+06	55.23					
359	100 ug/ml	5724.900000	3	1908.32	0.5	20	0.025	7.63E+04	10	7.63E+05	40.61	40.61	39.40	4.415459		100
	EL1 100 ug/ml Talc	5383.506000	3	1794.5	0.5	20	0.025	7.18E+04	10	7.18E+05	38.19					
		4517.519127	3	1505.84	0.5	20	0.025	6.02E+04	10	6.02E+05	32.05					
383	Normal Ovarian Unt 72 hr	15105.982000	3	5035.33	0.5	20	0.025	2.01E+05	10	2.01E+06	107.16	43.90	105.26	1.01558		
		15269.367000	3	5089.46	0.5	20	0.025	2.04E+05	10	2.04E+06	108.48					
		15388.223000	3	5129.41	0.5	20	0.025	2.05E+05	10	2.05E+06	109.16					
384	5 ug/ml	1877.231000	3	625.744	0.5	20	0.025	2.50E+04	10	2.50E+05	13.32	7.77	13.00	0.982279		
		1789.221000	3	596.407	0.5	20	0.025	2.39E+04	10	2.39E+05	12.69					
		1887.099000	3	629.033	0.5	20	0.025	2.52E+04	10	2.52E+05	13.39					
385	20 ug/ml	888.982000	3	296.327	0.5	20	0.025	1.19E+04	10	1.19E+05	6.31	3.16	6.06	0.287066		
		672.091000	3	290.697	0.5	20	0.025	1.16E+04	10	1.16E+05	6.19					
		811.991000	3	270.664	0.5	20	0.025	1.08E+04	10	1.08E+05	5.76					
386	100 ug/ml	677.928000	3	225.976	0.5	20	0.025	9.04E+03	10	9.04E+04	4.81	1.91	4.49	0.380961		
		599.590000	3	199.863	0.5	20	0.025	7.99E+03	10	7.99E+04	4.25					
		622.981000	3	207.66	0.5	20	0.025	8.31E+03	10	8.31E+04	4.42					
379	FT33 Unt 72 hr	9514.808000	3	3171.6	0.5	20	0.025	1.27E+05	10	1.27E+06	67.50	53.71	64.68	5.980631		
		8322.550000	3	2774.18	0.5	20	0.025	1.11E+05	10	1.11E+06	59.04					
		7579.535000	3	2559.85	0.5	20	0.025	1.02E+05	10	1.02E+06	54.48					
380	5 ug/ml Talc	6344.823000	3	2114.94	0.5	20	0.025	8.46E+04	10	8.46E+05	45.01	43.29	44.81	0.998061		
		6239.339000	3	2096.45	0.5	20	0.025	8.39E+04	10	8.39E+05	44.62					
		6555.990000	3	2185.33	0.5	20	0.025	8.74E+04	10	8.74E+05	46.51					
381	20 ug/ml Talc	4399.378000	3	1466.13	0.5	20	0.025	5.86E+04	10	5.86E+05	31.20	29.96	30.30	0.905928		
		4144.962000	3	1381.66	0.5	20	0.025	5.53E+04	10	5.53E+05	29.40					
		4299.378000	3	1433.13	0.5	20	0.025	5.73E+04	10	5.73E+05	30.50					
382	100 ug/ml Talc	554.139000	3	184.76	0.5	20	0.025	7.39E+03	10	7.39E+04	3.93	1.45	4.76	0.47783		
		672.991000	3	224.327	0.5	20	0.025	8.97E+03	10	8.97E+04	4.77					
		668.922000	3	222.974	0.5	20	0.025	8.92E+03	10	8.92E+04	4.75					
360	SKOV-3 Unt 72 hr	11178.499000	3	3726.17	0.5	20	0.025	1.49E+05	10	1.49E+06	79.30	67.92	74.40	4.246458		
		10130.117000	3	3376.71	0.5	20	0.025	1.35E+05	10	1.35E+06	71.86					
		10152.669000	3	3384.22	0.5	20	0.025	1.35E+05	10	1.35E+06	72.02					
361	5 ug/ml	717.629032	3	1720.58	0.5	20	0.025	6.88E+04	10	6.88E+05	36.62	31.14	34.66	2.900092		
		532.905251	3	1536.29	0.5	20	0.025	6.15E+04	10	6.15E+05	32.70					
		153.514711	3	1454.54	0.5	20	0.025	5.82E+04	10	5.82E+05	30.96					
362	20 ug/ml	982.200159	3	988.97	0.5	20	0.025	3.96E+04	10	3.96E+05	21.05	17.11	18.62	2.539827		
		587.421719	3	892.134	0.5	20	0.025	3.57E+04	10	3.57E+05	18.99					
		341.940073	3	743.908	0.5	20	0.025	2.97E+04	10	2.97E+05	15.81					
363	100 ug/ml	925.443693	3	308.481	0.5	20	0.025	1.23E+04	10	1.23E+05	6.57	6.29	4.67	0.190668		
		885.237250	3	295.412	0.5	20	0.025	1.18E+04	10	1.18E+05	6.29					
		163.813817	3	54.6045	0.5	20	0.025	2.18E+03	10	2.18E+04	1.16					
364	TOV112 Unt 72 hr	9774.836000	3	3258.27	0.5	20	0.025	1.30E+05	10	1.30E+06	69.34	36.77	66.97	5.027599		
		8772.540000	3	2924.18	0.5	20	0.025	1.17E+05	10	1.17E+06	62.23					
		7079.596000	3	2359.87	0.5	20	0.025	9.44E+04	10	9.44E+05	50.22					
365	TOV112 5 ug/ml Talc	6450.657000	3	2150.22	0.5	20	0.025	8.60E+04	10	8.60E+05	45.76	22.29	45.39	0.723278		
		6345.112000	3	2115.04	0.5	20	0.025	8.46E+04	10	8.46E+05	45.01					
		6548.981000	3	2182.99	0.5	20	0.025	8.73E+04	10	8.73E+05	46.46					
366	TOV112 20 ug/ml Talc	4355.817000	3	1452.27	0.5	20	0.025	5.81E+04	10	5.81E+05	30.91	29.26	30.09	0.834916		
		4125.060000	3	1375.02	0.5	20	0.025	5.50E+04	10	5.50E+05	29.26					
		4205.280000	3	1401.76	0.5	20	0.025	5.61E+04	10	5.61E+05	29.83					
367	TOV112 100 ug/ml Talc	468.004468	3	156.001	0.5	20	0.025	6.24E+03	10	6.24E+04	3.32	0.80	3.88	2.005517		
		368.040241	3	89.3467	0.5	20	0.025	3.57E+03	10	3.57E+04	1.90					
		826.037950	3	275.346	0.5	20	0.025	1.10E+04	10	1.10E+05	5.86					
368	A2780 Unt 72 hr	13197.089000	3	4399.7	0.5	20	0.025	1.76E+05	10	1.76E+06	93.55	78.53	82.70	4.480384		
		11210.870000	3	3736.96	0.5	20	0.025	1.49E+05	10	1.49E+06	79.53					
		12105.843000	3	4035.28	0.5	20	0.025	1.61E+05	10	1.61E+06	85.88					
369	5 ug/ml	7785.982000	3	2595.33	0.5	20	0.025	1.04E+05	10	1.04E+06	55.23	32.21	59.45	5.340978		
		9195.282000	3	3065.76	0.5	20	0.025	1.22E+05	10	1.22E+06	65.16					
		8973.241000	3	2991.08	0.5	20	0.025	1.20E+05	10	1.20E+06	63.98					
370	20 ug/ml	6524.891000	3	2174.96	0.5	20	0.025	8.70E+04	10	8.70E+05	46.29	26.64	41.78	4.542891		
		5247.982000	3	1749.33	0.5	20	0.025	7.00E+04	10	7.00E+05	37.23					
		5986.997000	3	1995.67	0.5	20	0.025	7.98E+04	10	7.98E+05	42.47					
371	100 ug/ml	1532.965000	3	510.855	0.5	20	0.025	2.04E+04	10	2.04E+05	10.87	3.77	9.82	0.068125		
		1238.764000	3	412.255	0.5	20	0.025	1.65E+04	10	1.65E+05	8.77					
		1250.345000	3	416.782	0.5	20	0.025	1.67E+04	10	1.67E+05	8.87					



SAED000014(color)

2/20/2008
Run qPCR GSR with samples 356 ~ 368

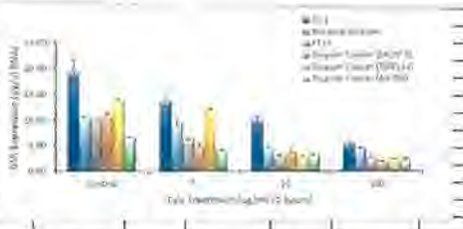
Run Summary	Smart Cycler 2.0d										
Run Name:	GSR 3ul 10x										
Std Curve:	GSR standard RADIANT SYBR										
Started At:	2/20/2008 11:00										
Number of Sites:	72										
Results Table:											
Site ID	Protocol	Sample ID	Sample Typ	Notes	Status	FAM Std/Res	FAM Ct	Cy3 Std/Res	Cy3 Ct	Melt Peak1	
B13	63-10	8	STD		OK	607000000	12.34	0	0	84.79	
B14	63-10	7	STD		OK	60700000	15.23	0	19.31	84.69	
B15	63-10	6	STD		OK	6070000	19.21	0	23.21	84.87	
B16	63-10	5	STD		OK	607000	23.72	0	27.87	84.99	
C1	63-10	4	STD		OK	60700	28.68	0	0	84.1	
C2	63-10	3	STD		OK	6070	33.61	0	36.93	84.36	
C3	63-10	2	STD		OK	607	37.13	0	0	84.39	
A1	GSR - RADIANT SYBR 2017	356	UNKN		OK	12491697.33	18.59	ND	26.21	84.49	
A2	GSR - RADIANT SYBR 2017		UNKN		OK	13197959.25	18.48	ND	26.88	84.68	
A3	GSR - RADIANT SYBR 2017		UNKN		OK	16497579.85	18.07	ND	0	84.77	
A4	GSR - RADIANT SYBR 2017	357	UNKN		OK	12550060.22	18.58	ND	26.22	84.84	
A5	GSR - RADIANT SYBR 2017		UNKN		OK	11465722.02	18.75	ND	27.6	84.7	
A6	GSR - RADIANT SYBR 2017		UNKN		OK	6176688.05	19.91	ND	0	84.44	
A7	GSR - RADIANT SYBR 2017	358	UNKN		OK	1215145.558	22.97	ND	28.58	84.72	
A8	GSR - RADIANT SYBR 2017		UNKN		OK	1332012.203	22.80	ND	28.73	84.76	
A9	GSR - RADIANT SYBR 2017		UNKN		OK	1119616.004	23.13	ND	27.76	84.52	
A10	GSR - RADIANT SYBR 2017	359	UNKN		OK	747389.005	23.69	ND	28.25	84.63	
A11	GSR - RADIANT SYBR 2017		UNKN		OK	674840.12	24.08	ND	27.91	84.72	
A12	GSR - RADIANT SYBR 2017		UNKN		OK	613358.594	23.73	ND	27.85	84.76	
A13	GSR - RADIANT SYBR 2017	360	UNKN		OK	1630605.248	22.42	ND	29.86	84.53	
A14	GSR - RADIANT SYBR 2017		UNKN		OK	1582898.701	22.47	ND	25.82	84.64	
A15	GSR - RADIANT SYBR 2017		UNKN		OK	1475190.908	22.61	ND	25.77	84.62	
A16	GSR - RADIANT SYBR 2017	361	UNKN		OK	1533631.102	22.53	ND	26.35	84.6	
B1	GSR - RADIANT SYBR 2017		UNKN		OK	1381068.599	22.73	ND	26.41	84.74	
B2	GSR - RADIANT SYBR 2017		UNKN		OK	994223.617	23.35	ND	26.52	84.63	
B3	GSR - RADIANT SYBR 2017	362	UNKN		OK	1232346.22	22.94	ND	26.4	84.68	
B4	GSR - RADIANT SYBR 2017		UNKN		OK	1454072.785	22.63	ND	26.83	84.85	
B5	GSR - RADIANT SYBR 2017		UNKN		OK	1604451.237	22.45	ND	26.53	84.93	
B6	GSR - RADIANT SYBR 2017	363	UNKN		OK	1313932.919	22.82	ND	27.46	84.73	
B7	GSR - RADIANT SYBR 2017		UNKN		OK	1243345.963	22.93	ND	27.74	84.79	
B8	GSR - RADIANT SYBR 2017		UNKN		OK	1120583.93	23.12	ND	26.84	84.7	
B9	GSR - RADIANT SYBR 2017	364	UNKN		OK	832867.79	23.68	ND	0	84.69	
B10	GSR - RADIANT SYBR 2017		UNKN		OK	904011.633	23.53	ND	26.53	84.77	
B11	GSR - RADIANT SYBR 2017		UNKN		OK	838919.217	23.67	ND	29.63	84.68	
B12	GSR - RADIANT SYBR 2017	365	UNKN		OK	911978.592	23.51	ND	28.56	84.92	
B13	GSR - RADIANT SYBR 2017		UNKN		OK	890503.785	23.56	ND	28.52	84.76	
B14	GSR - RADIANT SYBR 2017		UNKN		OK	924489.784	23.49	ND	29.83	84.66	
B15	GSR - RADIANT SYBR 2017	366	UNKN		OK	892768.919	23.55	ND	27.36	84.68	
B16	GSR - RADIANT SYBR 2017		UNKN		OK	903235.028	23.53	ND	28.53	84.76	
C1	GSR - RADIANT SYBR 2017		UNKN		OK	858318.882	23.62	ND	27.95	84.85	
C2	GSR - RADIANT SYBR 2017	367	UNKN		OK	937151.754	23.46	ND	27.95	84.91	
C3	GSR - RADIANT SYBR 2017		UNKN		OK	914716.164	23.51	ND	27.79	84.79	
C4	GSR - RADIANT SYBR 2017		UNKN		OK	955679.539	23.42	ND	27.73	84.67	
C5	GSR - RADIANT SYBR 2017	368	UNKN		OK	1630605.248	23.53	ND	27.87	84.82	
C6	GSR - RADIANT SYBR 2017		UNKN		OK	1582898.701	23.39	ND	27.68	84.77	
C7	GSR - RADIANT SYBR 2017		UNKN		OK	1475190.908	23.38	ND	27.67	84.78	
C8	GSR - RADIANT SYBR 2017	369	UNKN		OK	1533631.102	25.10	ND	29.49	84.81	
C9	GSR - RADIANT SYBR 2017		UNKN		OK	1381068.599	23.27	ND	27.64	84.95	
C10	GSR - RADIANT SYBR 2017		UNKN		OK	994223.617	23.41	ND	27.45	84.91	
C11	GSR - RADIANT SYBR 2017	370	UNKN		OK	1232346.22	22.92	ND	0	84.83	
C12	GSR - RADIANT SYBR 2017		UNKN		OK	1454072.785	23.36	ND	27.92	84.81	
C13	GSR - RADIANT SYBR 2017		UNKN		OK	1604451.237	23.50	ND	27.31	84.83	
A1	GSR - RADIANT SYBR 2017	371	UNKN		OK	1313932.919	23.49	ND	28.64	84.51	
A2	GSR - RADIANT SYBR 2017		UNKN		OK	1243345.963	23.73	ND	27.83	84.64	
A3	GSR - RADIANT SYBR 2017		UNKN		OK	1120583.93	23.16	ND	0	84.64	
A4	GSR - RADIANT SYBR 2017	379	UNKN		OK	832867.79	23.68	ND	28.35	84.71	
A5	GSR - RADIANT SYBR 2017		UNKN		OK	904011.633	23.53	ND	27.68	84.63	
A6	GSR - RADIANT SYBR 2017		UNKN		OK	838919.217	23.67	ND	27.53	84.71	
A7	GSR - RADIANT SYBR 2017	380	UNKN		OK	911978.592	23.51	ND	28.61	84.7	
A8	GSR - RADIANT SYBR 2017		UNKN		OK	890503.785	23.56	ND	27.34	84.74	
A9	GSR - RADIANT SYBR 2017		UNKN		OK	924489.784	23.49	ND	27.56	84.37	
A10	GSR - RADIANT SYBR 2017	381	UNKN		OK	892768.919	23.55	ND	27.52	84.7	
A11	GSR - RADIANT SYBR 2017		UNKN		OK	903235.028	23.53	ND	0	84.39	
A12	GSR - RADIANT SYBR 2017		UNKN		OK	858318.882	23.62	ND	27.7	84.7	
A13	GSR - RADIANT SYBR 2017	382	UNKN		OK	937151.754	23.46	ND	27.5	84.57	
A14	GSR - RADIANT SYBR 2017		UNKN		OK	914716.164	23.51	ND	25.59	84.49	
A15	GSR - RADIANT SYBR 2017		UNKN		OK	955679.539	23.42	ND	25.41	84.5	
A16	GSR - RADIANT SYBR 2017	383	UNKN		OK	900688.733	22.42	ND	26.81	84.62	
B1	GSR - RADIANT SYBR 2017		UNKN		OK	971821.883	22.47	ND	0	84.75	
B2	GSR - RADIANT SYBR 2017		UNKN		OK	975332.42	22.81	ND	26.55	84.79	
B3	GSR - RADIANT SYBR 2017	384	UNKN		OK	991545.027	22.53	ND	0	84.57	
B4	GSR - RADIANT SYBR 2017		UNKN		OK	1036721.604	22.73	ND	0	84.69	
B5	GSR - RADIANT SYBR 2017		UNKN		OK	964544.979	23.35	ND	27.82	84.78	
B6	GSR - RADIANT SYBR 2017	385	UNKN		OK	1248521.587	22.94	ND	28.33	84.74	
B7	GSR - RADIANT SYBR 2017		UNKN		OK	989162.626	22.63	ND	28.7	84.67	
B8	GSR - RADIANT SYBR 2017		UNKN		OK	915563.788	22.45	ND	0	84.75	
B9	GSR - RADIANT SYBR 2017	386	UNKN		OK	922938.923	22.82	ND	29.31	84.83	
B10	GSR - RADIANT SYBR 2017		UNKN		OK	813697.526	22.93	ND	28.72	84.85	
B11	GSR - RADIANT SYBR 2017		UNKN		OK	1096341.307	23.12	ND	28.62	84.71	

Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp	extension time (s) and temp
60	15	10, 59	30, 72

Primer information & Calculation

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product /Amplicon Length	Start Position
NM_000637	GSR	AAT CTC ACC AAG TCC CAT ATA GAA ATC ATC CGT GGC CAT AAG CCC ACA ATA GAG GTC AGT GGG AAA AAG TAC ACC GCC CCA CAC ATC CTG ATC GCC ACA GGT G	TCACCAAGTCCCATATAGAAATC	TGTGGCGATCAGGATGTG	103	116	

Gene of Interest	GSR		
		Units	Formula
Daltons = 1660.24 grams	1660.24	g	
Mass of Base pair	615	Da	
Avg. Mass/base	10525	Da	
Length of entire gene	615	bases	
Mass in Daltons	1.14E-04	Da	= number bases x avg. mass/base
Mass in grams	5.22E-20	g	= mass in Da x mass of 1 Da in grams
Mass in ug	5.22E-14	ug	= above / 10E-6
Mass in ng	5.22E-11	ng/copy	= above x 10E-3



ID	Sample	Copy #	ul cDNA used	copies/ul cDNA	ug RNA used	ul cDNA made	ug RNA/ul cDNA	copies/ug RNA	Dilution Factor	Copies/ug RNA x Df	pg/pg RNA	Normalized	Average	St Dev
355	EL1 Unt 72 hr	2491697.3	3	830565.78	0.5	20	0.025	3.32E+07	10	3.32E+08	17.34	16.89	19.25	2.360123
		3187855.3	3	1062618.4	0.5	20	0.025	4.25E+07	10	4.25E+08	22.18	21.62		
		6497579.9	2	2165860	0.5	20	0.025	8.66E+07	10	8.66E+08	45.22	44.06		
357	EL1 5 ug/ml Talc	2550060.2	3	850020.07	0.5	20	0.025	3.40E+07	10	3.40E+08	17.75	17.42	19.71	1.275183
		1465722	3	488574.01	0.5	20	0.025	1.95E+07	10	1.95E+08	10.20	10.01		
		2176698.1	3	725562.68	0.5	20	0.025	2.90E+07	10	2.90E+08	15.15	14.87		
358	EL1 20 ug/ml Talc	1215145.6	3	405048.52	0.5	20	0.025	1.62E+07	10	1.62E+08	8.46	10.61	10.19	0.58981
		1332012.2	3	444004.07	0.5	20	0.025	1.78E+07	10	1.78E+08	9.27	11.63		
		1119616	3	373205.33	0.5	20	0.025	1.49E+07	10	1.49E+08	7.78	9.78		
359	EL1 100 ug/ml Talc	747389.01	3	249129.67	0.5	20	0.025	9.97E+06	10	9.97E+07	5.20	5.20	5.43	0.324816
		674840.12	3	224946.71	0.5	20	0.025	9.00E+06	10	9.00E+07	4.70	4.70		
		813358.59	3	271119.53	0.5	20	0.025	1.08E+07	10	1.08E+08	5.66	5.66		
383	Nokmd Bivalve Unt 72 hr	1630605.2	3	543535.08	0.5	20	0.025	2.17E+07	10	2.17E+08	11.35	10.72	10.57	0.221873
		1582898.7	3	527632.9	0.5	20	0.025	2.11E+07	10	2.11E+08	11.02	10.41		
		1475190.9	3	491730.3	0.5	20	0.025	1.97E+07	10	1.97E+08	10.27	9.70		
384	5 ug/ml	1533631.1	3	511210.37	0.5	20	0.025	2.04E+07	10	2.04E+08	10.67	10.17	9.68	0.715034
		1381068.6	3	460356.2	0.5	20	0.025	1.84E+07	10	1.84E+08	9.61	9.15		
		994223.62	3	331407.87	0.5	20	0.025	1.33E+07	10	1.33E+08	6.92	6.59		
385	20 ug/ml	1232346.2	3	410782.07	0.5	20	0.025	1.64E+07	5	8.22E+07	4.29	3.86	4.79	0.933967
		1454072.8	3	484690.93	0.5	20	0.025	1.94E+07	5	9.69E+07	5.06	4.56		
		1604451.2	3	534817.08	0.5	20	0.025	2.14E+07	5	1.07E+08	5.58	5.03		
386	100 ug/ml	1313932.9	3	437977.64	0.5	20	0.025	1.75E+07	5	8.76E+07	4.57	4.57	4.45	0.125989
		1243346	3	414448.65	0.5	20	0.025	1.66E+07	5	8.29E+07	4.33	4.33		
		1120583.9	3	373527.98	0.5	20	0.025	1.49E+07	5	7.47E+07	3.90	3.90		
379	FT31 Unt 72 hr	832867.79	3	277622.6	0.5	20	0.025	1.11E+07	30	3.33E+08	17.39	10.27	10.83	0.652777
		904011.83	3	301337.28	0.5	20	0.025	1.21E+07	30	3.62E+08	18.87	11.15		
		838919.22	3	279639.74	0.5	20	0.025	1.12E+07	30	3.36E+08	17.51	10.35		
380	5 ug/ml Talc	911978.59	3	303992.86	0.5	20	0.025	1.22E+07	20	2.43E+08	12.69	6.29	6.21	0.104856
		890503.79	3	296834.6	0.5	20	0.025	1.19E+07	20	2.37E+08	12.39	6.14		
		924489.78	3	308163.26	0.5	20	0.025	1.23E+07	20	2.47E+08	12.87	6.37		
381	20 ug/ml Talc	892768.92	3	297589.64	0.5	20	0.025	1.19E+07	5	5.95E+07	3.11	3.11	3.05	0.064759
		903235.03	3	301078.34	0.5	20	0.025	1.20E+07	5	6.02E+07	3.14	3.14		
		858316.88	3	286105.29	0.5	20	0.025	1.14E+07	5	5.72E+07	2.99	2.99		
382	100 ug/ml Talc	937151.75	3	312383.92	0.5	20	0.025	1.25E+07	10	1.25E+08	6.52	2.73	2.72	0.059714
		914716.16	3	304905.39	0.5	20	0.025	1.22E+07	10	1.22E+08	6.37	2.66		
		955679.54	3	318559.85	0.5	20	0.025	1.27E+07	10	1.27E+08	6.65	2.78		
380	BKDV-3 Unt 72 hr	1630605.2	3	543535.08	0.5	20	0.025	2.17E+07	10	2.17E+08	11.35	11.35	11.18	0.234749
		1582898.7	3	527632.9	0.5	20	0.025	2.11E+07	10	2.11E+08	11.02	11.02		
		1475190.9	3	491730.3	0.5	20	0.025	1.97E+07	10	1.97E+08	10.27	10.27		
381	5 ug/ml	1533631.1	3	511210.37	0.5	20	0.025	2.04E+07	10	2.04E+08	10.67	5.28	5.01	0.374169
		1381068.6	3	460356.2	0.5	20	0.025	1.84E+07	10	1.84E+08	9.61	4.75		
		994223.62	3	331407.87	0.5	20	0.025	1.33E+07	10	1.33E+08	6.92	3.42		
382	20 ug/ml	1232346.2	3	410782.07	0.5	20	0.025	1.64E+07	5	8.22E+07	4.29	3.07	3.81	0.284765
		1454072.8	3	484690.93	0.5	20	0.025	1.94E+07	5	9.69E+07	5.06	3.62		
		1604451.2	3	534817.08	0.5	20	0.025	2.14E+07	5	1.07E+08	5.58	3.99		
383	100 ug/ml	1313932.9	3	437977.64	0.5	20	0.025	1.75E+07	5	8.76E+07	4.57	1.97	1.91	0.073995
		1243346	3	414448.65	0.5	20	0.025	1.66E+07	5	8.29E+07	4.33	1.86		
		1120583.9	3	373527.98	0.5	20	0.025	1.49E+07	5	7.47E+07	3.90	1.68		
384	TGW112 Unt 72 hr	832867.79	3	277622.6	0.5	20	0.025	1.11E+07	30	3.33E+08	17.39	13.83	13.68	0.078883
		904011.83	3	301337.28	0.5	20	0.025	1.21E+07	30	3.62E+08	18.87	15.02		
		838919.22	3	279639.74	0.5	20	0.025	1.12E+07	30	3.36E+08	17.51	13.94		
385	TGW112 5 ug/ml Talc	911978.59	3	303992.86	0.5	20	0.025	1.22E+07	20	2.43E+08	12.69	12.21	12.06	0.203256
		890503.79	3	296834.6	0.5	20	0.025	1.19E+07	20	2.37E+08	12.39	11.92		
		924489.78	3	308163.26	0.5	20	0.025	1.23E+07	20	2.47E+08	12.87	12.37		
386	TGW112 20 ug/ml Talc	892768.92	3	297589.64	0.5	20	0.025	1.19E+07	5	5.95E+07	3.11	2.99	2.93	0.081331
		903235.03	3	301078.34	0.5	20	0.025	1.20E+07	5	6.02E+07	3.14	3.02		
		858316.88	3	286105.29	0.5	20	0.025	1.14E+07	5	5.72E+07	2.99	2.87		
387	TGW112 100 ug/ml Talc	937151.75	3	312383.92	0.5	20	0.025	1.25E+07	10	1.25E+08	6.52	2.41	2.41	0.052703
		914716.16	3	304905.39	0.5	20	0.025	1.22E+07	10	1.22E+08	6.37	2.35		
		955679.54	3	318559.85	0.5	20	0.025	1.27E+07	10	1.27E+08	6.65	2.46		
388	A2781104 72 hr	900868.73	3	300289.58	0.5	20	0.025	1.20E+07	10	1.20E+08	6.27	6.27	6.81	0.619274
		971821.88	3	323940.63	0.5	20	0.025	1.30E+07	10	1.30E+08	6.76	6.76		
		975332.42	3	325110.81	0.5	20	0.025	1.30E+07	10	1.30E+08	6.79	6.79		
389	5 ug/ml	391645.03	3	130548.34	0.5	20	0.025	5.22E+06	10	5.22E+07	2.73	1.59	4.67	0.213886
		1038721.6	3	346240.53	0.5	20	0.025	1.38E+07	10	1.38E+08	7.23	4.22		
		964544.98	3	321514.99	0.5	20	0.025	1.29E+07	10	1.29E+08	6.71	3.92		
390	20 ug/ml	1248521.6	3	416173.86	0.5	20	0.025	1.66E+07	10	1.66E+08	8.69	4.35	3.32	3.188329
		989162.63	3	329720.88	0.5	20	0.025	1.32E+07	10	1.32E+08	6.88	3.45		
		915563.77	3	305187.92	0.5	20	0.025	1.22E+07	10	1.22E+08	6.37	3.19		
391	100 ug/ml	922938.92	3	307646.31	0.5	20	0.025	1.23E+07	10	1.23E+08	6.42	2.56	2.49	0.21865
		813667.53	3	271222.51	0.5	20	0.025	1.08E+07	10	1.08E+08	5.66	2.25		
		1096341.3	3	365447.1	0.5	20	0.025	1.46E+07	10	1.46E+08	7.63	3.04		

SAED000016(color)

2/21/2018

Run PCR — iNOS with samples 356~368

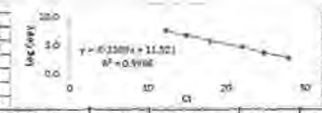
Primer information

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product /Amplicon Length	Start Position
NM_000625	iNOS Dec	GAGGACCACATCTACCAGGAGGAGATGCTGGAGATGG CCCAGAAGGGGGTGCTGCATGCGGTGCACACAGCCTAT TCCCGCCTGCCTGG	GAGGACCACATCTACCAGGA	CCAGGCAGGCGGGAATAG	89	89	3325

Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp	extension time (s) and temp
60	15	10,64	30, 72

Raw data

Run Summary (Smart Cycler 2.0d)
Run Name: iNOS Std 10x
Std Curve: new iNOS stand RAD 09-10 7to5x
Started: 2/21/2018 12:10
Number: 72



Results Table

Site ID	Protocol	Sample ID	Sample Type	Notes	Status	FAM Std/R	FAM Ct	Cy3 Std/Res	Cy3 Ct	TxR Std/Res	TxR Ct	Cy5 Std/Res	Cy5 Ct	Melt Peak1	Y-Log Copy
C9	iNOS RADIANT SYBR 2017	7	STD		OK	81500000	12.38	0	18.89	0	0	0	0	88.47	7.8
C10	iNOS RADIANT SYBR 2017	6	STD		OK	8150000	14.94	0	18.64	0	0	0	0	88.2	6.8
C11	iNOS RADIANT SYBR 2017	5	STD		OK	815000	17.97	0	0	0	0	0	0	88.36	5.8
C12	iNOS RADIANT SYBR 2017	4	STD		OK	81500	22.07	0	26.97	0	0	0	0	88.37	4.8
C13	iNOS RADIANT SYBR 2017	3	STD		OK	8150	24.67	0	28.34	0	0	0	0	88.32	3.8
C14	iNOS RADIANT SYBR 2017	2	STD		OK	815	28.04	0	31.82	0	0	0	0	88.34	2.8
A1	iNOS RADIANT SYBR 2017	356	UNKN		OK	550 436	28.22	ND	31.19	ND		0	ND	0	85.98
A2	iNOS RADIANT SYBR 2017		UNKN		OK	662 973	26.21	ND	31.95	ND		0	ND	0	88.25
A3	iNOS RADIANT SYBR 2017		UNKN		OK	824 104	28.07	ND	0	ND		0	ND	0	88.29
A4	iNOS RADIANT SYBR 2017	357	UNKN		OK	1763 75	26.81	ND	31.51	ND		0	ND	0	88.16
A5	iNOS RADIANT SYBR 2017		UNKN		OK	1638 771	26.72	ND	32.64	ND		0	ND	0	88.24
A6	iNOS RADIANT SYBR 2017		UNKN		OK	1605 088	26.75	ND	31.85	ND		0	ND	0	88.1
A7	iNOS RADIANT SYBR 2017	358	UNKN		OK	2532 63	25.11	ND	33.61	ND		0	ND	0	88.31
A8	iNOS RADIANT SYBR 2017		UNKN		OK	2620 278	26.06	ND	32.18	ND		0	ND	0	88.32
A9	iNOS RADIANT SYBR 2017		UNKN		OK	2797 271	26.62	ND	32.13	ND		0	ND	0	88.84
A10	iNOS RADIANT SYBR 2017	359	UNKN		OK	3441 472	25.08	ND	30.15	ND		0	ND	0	88.26
A11	iNOS RADIANT SYBR 2017		UNKN		OK	3606 513	25.01	ND	30.53	ND		0	ND	0	88.28
A12	iNOS RADIANT SYBR 2017		UNKN		OK	8779 661	23.55	ND	30.42	ND		0	ND	0	88.17
A13	iNOS RADIANT SYBR 2017	360	UNKN		OK	1195 884	27.15	ND	33.97	ND		0	ND	0	88.28
A14	iNOS RADIANT SYBR 2017		UNKN		OK	1212 564	27.14	ND	32.77	ND		0	ND	0	88.13
A15	iNOS RADIANT SYBR 2017		UNKN		OK	1181 64	27.26	ND	33.97	ND		0	ND	0	88.03
A16	iNOS RADIANT SYBR 2017	361	UNKN		OK	2173 64	26.32	ND	33.36	ND		0	ND	0	88.16
B1	iNOS RADIANT SYBR 2017		UNKN		OK	2188 53	26.31	ND	0	ND		0	ND	0	88.35
B2	iNOS RADIANT SYBR 2017		UNKN		OK	2121 36	26.36	ND	33.8	ND		0	ND	0	88.31
B3	iNOS RADIANT SYBR 2017	362	UNKN		OK	3598 51	25.62	ND	0	ND		0	ND	0	88.25
B4	iNOS RADIANT SYBR 2017		UNKN		OK	3460 30	25.67	ND	34.69	ND		0	ND	0	88.21
B5	iNOS RADIANT SYBR 2017		UNKN		OK	3740 39	25.58	ND	34.54	ND		0	ND	0	88.31
B6	iNOS RADIANT SYBR 2017	363	UNKN		OK	4008 248	25.45	ND	31.59	ND		0	ND	0	88.41
B7	iNOS RADIANT SYBR 2017		UNKN		OK	4052 365	25.17	ND	31.94	ND		0	ND	0	88.17
B8	iNOS RADIANT SYBR 2017		UNKN		OK	4028 142	25.46	ND	33.93	ND		0	ND	0	88.19
B9	iNOS RADIANT SYBR 2017	364	UNKN		OK	984 502	27.42	ND	32.67	ND		0	ND	0	88.17
B10	iNOS RADIANT SYBR 2017		UNKN		OK	942 888	27.49	ND	31.77	ND		0	ND	0	88.36
B11	iNOS RADIANT SYBR 2017		UNKN		OK	1013 606	27.39	ND	32.72	ND		0	ND	0	88.2
B12	iNOS RADIANT SYBR 2017	365	UNKN		OK	2459 062	26.16	ND	32.69	ND		0	ND	0	88.38
B13	iNOS RADIANT SYBR 2017		UNKN		OK	21247 16	23.14	ND	31.75	ND		0	ND	0	88.26
B14	iNOS RADIANT SYBR 2017		UNKN		OK	2644 139	26.05	ND	0	ND		0	ND	0	88.21
B15	iNOS RADIANT SYBR 2017	366	UNKN		OK	2051 07	26.40	ND	31.11	ND		0	ND	0	88.2
B16	iNOS RADIANT SYBR 2017		UNKN		OK	2051 872	26.43	ND	31.77	ND		0	ND	0	88.14
C1	iNOS RADIANT SYBR 2017		UNKN		OK	2191 008	26.31	ND	31.62	ND		0	ND	0	88.33
C2	iNOS RADIANT SYBR 2017	367	UNKN		OK	7818 698	24.53	ND	31.75	ND		0	ND	0	88.41
C3	iNOS RADIANT SYBR 2017		UNKN		OK	7029 289	24.66	ND	31.65	ND		0	ND	0	88.26
C4	iNOS RADIANT SYBR 2017		UNKN		OK	7293 577	24.64	ND	31.59	ND		0	ND	0	88.42
C5	iNOS RADIANT SYBR 2017	368	UNKN		OK	853 554	27.68	ND	31.77	ND		0	ND	0	88.23
C6	iNOS RADIANT SYBR 2017		UNKN		OK	880 02	27.44	ND	31.52	ND		0	ND	0	88.29
C7	iNOS RADIANT SYBR 2017		UNKN		OK	907 700	27.54	ND	31.56	ND		0	ND	0	88.3
C8	iNOS RADIANT SYBR 2017	369	UNKN		OK	2907 309	25.92	ND	32.86	ND		0	ND	0	88.31
C9	iNOS RADIANT SYBR 2017		UNKN		OK	2771 779	26.01	ND	31.88	ND		0	ND	0	88.35
C10	iNOS RADIANT SYBR 2017		UNKN		OK	2724 242	26.01	ND	31.74	ND		0	ND	0	88.3
C11	iNOS RADIANT SYBR 2017	370	UNKN		OK	3991 927	25.47	ND	0	ND		0	ND	0	88.3
C12	iNOS RADIANT SYBR 2017		UNKN		OK	3634 487	25.60	ND	32.39	ND		0	ND	0	88.25
C13	iNOS RADIANT SYBR 2017		UNKN		OK	3748 079	25.56	ND	31.5	ND		0	ND	0	88.16
C14	iNOS RADIANT SYBR 2017	371	UNKN		OK	8451 56	24.43	ND	33.8	ND		0	ND	0	88.29
C15	iNOS RADIANT SYBR 2017		UNKN		OK	8687 2	24.39	ND	34.8	ND		1	ND	0	88.29
C16	iNOS RADIANT SYBR 2017		UNKN		OK	8870 27	24.36	ND	35.9	ND		2	ND	0	88.29
A4	iNOS RADIANT SYBR 2017	379	UNKN		OK	998 221	27.41	ND	31.39	ND		0	ND	0	88.11
A5	iNOS RADIANT SYBR 2017		UNKN		OK	982 022	27.43	ND	32.83	ND		0	ND	0	88.31
A6	iNOS RADIANT SYBR 2017		UNKN		OK	1011 299	27.39	ND	31.68	ND		0	ND	0	88
A7	iNOS RADIANT SYBR 2017	380	UNKN		OK	2450 062	26.16	ND	31.59	ND		0	ND	0	88.29
A8	iNOS RADIANT SYBR 2017		UNKN		OK	2324 18	26.23	ND	30.37	ND		0	ND	0	88.2
A9	iNOS RADIANT SYBR 2017		UNKN		OK	2544 139	26.10	ND	30.53	ND		0	ND	0	88.36
A10	iNOS RADIANT SYBR 2017	381	UNKN		OK	2131 085	26.35	ND	30.34	ND		0	ND	0	88.2
A11	iNOS RADIANT SYBR 2017		UNKN		OK	2251 456	26.27	ND	30.29	ND		0	ND	0	88.09
A12	iNOS RADIANT SYBR 2017		UNKN		OK	2151 55	26.33	ND	30.49	ND		0	ND	0	88.19
A13	iNOS RADIANT SYBR 2017	382	UNKN		OK	7560 934	24.58	ND	33.65	ND		0	ND	0	88.13
A14	iNOS RADIANT SYBR 2017		UNKN		OK	7252 791	24.55	ND	32.4	ND		0	ND	0	88.11
A15	iNOS RADIANT SYBR 2017		UNKN		OK	7423 022	24.57	ND	31.81	ND		0	ND	0	88.2
A16	iNOS RADIANT SYBR 2017	383	UNKN		OK	1044 584	27.35	ND	33.29	ND		0	ND	0	88.14
B1	iNOS RADIANT SYBR 2017		UNKN		OK	1103 832	27.27	ND	0	ND		0	ND	0	88.31
B2	iNOS RADIANT SYBR 2017		UNKN		OK	1115 821	27.25	ND	33.56	ND		0	ND	0	88.33
B3	iNOS RADIANT SYBR 2017	384	UNKN		OK	1911 298	26.50	ND	0	ND		0	ND	0	88.22
B4	iNOS RADIANT SYBR 2017		UNKN		OK	1672 882	26.53	ND	33.71	ND		0	ND	0	88.24
B5	iNOS RADIANT SYBR 2017		UNKN		OK	1782 822	26.60	ND	33.58	ND		0	ND	0	88.32
B6	iNOS RADIANT SYBR 2017	385	UNKN		OK	2780 221	25.96	ND	31.71	ND		0	ND	0	88.25
B7	iNOS RADIANT SYBR 2017		UNKN		OK	2891 822	25.93	ND	32.63	ND		0	ND	0	88.15
B8	iNOS RADIANT SYBR 2017		UNKN		OK	2777 119	25.98	ND	0	ND		0	ND	0	88.26
B9	iNOS RADIANT SYBR 2017	386	UNKN		OK	4177 939	25.41	ND	32.58	ND		0	ND	0	88.33
B10	iNOS RADIANT SYBR 2017		UNKN		OK	4522 782	25.30	ND	32.15	ND		0	ND	0	88.35
B11	iNOS RADIANT SYBR 2017		UNKN		OK	4439 963	25.33	ND	32.68	ND		0	ND	0	88.07

SAED000017(color)

Calculation

Gene of Interest	EIF3C													
		Unit	Formula											
1 Dalton = 1.66E-24 grams	1.66E-24	g												
Mass of basic pair	615	Da												
Avg Mass/base	305.25	Da												
Length of entire gene	89	bases												
Mass in Daltons	2.72E+04	Da	= number bases x avg mass/base											
Mass in grams	4.51E-20	g	= mass in Da x mass of a Da in grams											
Mass in ug	4.51E-14	ug	=above / 10E-6											
Mass in ng	4.51E-11	ng/copy	= above x 10E3											
#####														
ID	Sample	Copy #	ul cDNA used	copies/ul cDNA	ug RNA used	ul cDNA made	ug RNA/ul cDNA	copies/ug RNA	Dilution Factor	Copies/ug RNA x DF	fg/ug RNA	Normalized	Average	St Dev
356	EL1 Unt T2 hr	560.436	3	186.812	0.5	20	0.025	7.47E+03	10	7.47E+04	3.37	3.28	3.48	0.253179
		562.873	3	187.62433	0.5	20	0.025	7.50E+03	10	7.50E+04	3.38	3.30		
		624.134	3	208.04467	0.5	20	0.025	8.32E+03	10	8.32E+04	3.75	3.66		
357	EL1 5 ug/ml Talc	1763.75	3	587.91667	0.5	20	0.025	2.35E+04	10	2.35E+05	10.61	10.41	10.04	0.531657
		1638.771	3	546.257	0.5	20	0.025	2.19E+04	10	2.19E+05	9.85	9.67		
		1605.089	3	535.02967	0.5	20	0.025	2.14E+04	10	2.14E+05	9.65	9.47		
358	EL1 20 ug/ml Talc	2532.63	3	844.21	0.5	20	0.025	3.38E+04	10	3.38E+05	15.23	19.11	19.44	0.461554
		2620.276	3	873.42533	0.5	20	0.025	3.49E+04	10	3.49E+05	15.76	19.77		
		2707.271	3	902.42367	0.5	20	0.025	3.61E+04	10	3.61E+05	16.28	20.43		
359	EL1 100 ug/ml Talc	3441.472	3	1147.1573	0.5	20	0.025	4.59E+04	10	4.59E+05	20.69	20.69	21.20	0.71061
		3608.612	3	1202.8707	0.5	20	0.025	4.81E+04	10	4.81E+05	21.70	21.70		
		3779.663	3	1259.8877	0.5	20	0.025	5.04E+04	10	5.04E+05	22.73	22.73		
383	Human Osteoblast Unt T2 hr	1044.584	3	348.19467	0.5	20	0.025	1.39E+04	10	1.39E+05	6.28	5.94	6.51	0.64811
		1103.932	3	367.97733	0.5	20	0.025	1.47E+04	10	1.47E+05	6.64	6.27		
		1115.921	3	371.97367	0.5	20	0.025	1.49E+04	10	1.49E+05	6.71	6.34		
384	5 ug/ml	1911.298	3	637.09933	0.5	20	0.025	2.55E+04	10	2.55E+05	11.49	10.95	10.64	0.55507
		1872.882	3	624.294	0.5	20	0.025	2.50E+04	10	2.50E+05	11.26	10.73		
		1782.822	3	594.274	0.5	20	0.025	2.38E+04	10	2.38E+05	10.72	10.21		
385	20 ug/ml	2780.221	3	926.74033	0.5	20	0.025	3.71E+04	10	3.71E+05	16.72	15.06	15.87	0.42711
		2891.922	3	963.974	0.5	20	0.025	3.86E+04	10	3.86E+05	17.39	15.67		
		2777.1193	3	925.70643	0.5	20	0.025	3.70E+04	10	3.70E+05	16.70	15.05		
386	100 ug/ml	4177.9387	3	1392.6462	0.5	20	0.025	5.57E+04	10	5.57E+05	25.12	25.12	26.04	1.69044
		4522.7821	3	1507.594	0.5	20	0.025	6.03E+04	10	6.03E+05	27.20	27.20		
		4439.992	3	1479.9973	0.5	20	0.025	5.92E+04	10	5.92E+05	26.70	26.70		
378	FTSk Unt T2 hr	998.221	3	332.74033	0.5	20	0.025	1.33E+04	10	1.33E+05	6.00	3.55	3.57	0.03287
		982.022	3	327.34067	0.5	20	0.025	1.31E+04	10	1.31E+05	5.90	3.49		
		1011.299	3	337.09967	0.5	20	0.025	1.35E+04	10	1.35E+05	6.08	3.59		
380	5 ug/ml Talc	2450.062	3	816.68733	0.5	20	0.025	3.27E+04	10	3.27E+05	14.73	7.30	7.44	0.13611
		2324.16	3	774.72	0.5	20	0.025	3.10E+04	10	3.10E+05	13.95	6.92		
		2544.139	3	848.04633	0.5	20	0.025	3.39E+04	10	3.39E+05	15.30	7.58		
381	20 ug/ml Talc	2131.035	3	710.345	0.5	20	0.025	2.84E+04	10	2.84E+05	12.81	12.81	13.12	0.17801
		2251.456	3	750.48533	0.5	20	0.025	3.00E+04	10	3.00E+05	13.54	13.54		
		2161.65	3	720.55	0.5	20	0.025	2.88E+04	10	2.88E+05	13.00	13.00		
382	100 ug/ml Talc	7560.934	3	2520.3113	0.5	20	0.025	1.01E+05	10	1.01E+06	45.46	19.02	19.39	0.39001
		7752.091	3	2584.0303	0.5	20	0.025	1.03E+05	10	1.03E+06	46.61	19.50		
		7623.022	3	2541.0073	0.5	20	0.025	1.02E+05	10	1.02E+06	45.84	19.17		
360	EHDV-3 Unt T2 hr	1198.584	3	399.528	0.5	20	0.025	1.60E+04	10	1.60E+05	7.21	7.21	7.14	0.2166
		1212.664	3	404.22133	0.5	20	0.025	1.62E+04	10	1.62E+05	7.29	7.29		
		1161.64	3	387.21333	0.5	20	0.025	1.55E+04	10	1.55E+05	6.98	6.98		
361	5 ug/ml	2173.64	3	724.54667	0.5	20	0.025	2.90E+04	10	2.90E+05	13.07	6.46	6.48	0.02121
		2186.53	3	728.84333	0.5	20	0.025	2.92E+04	10	2.92E+05	13.15	6.50		
		2121.38	3	707.12667	0.5	20	0.025	2.83E+04	10	2.83E+05	12.76	6.31		
362	20 ug/ml	3598.51	3	1199.5033	0.5	20	0.025	4.80E+04	10	4.80E+05	21.64	15.48	15.23	0.35861
		3480.36	3	1160.12	0.5	20	0.025	4.64E+04	10	4.64E+05	20.93	14.97		
		3740.39	3	1246.7967	0.5	20	0.025	4.99E+04	10	4.99E+05	22.49	16.09		
363	100 ug/ml	4066.248	3	1355.416	0.5	20	0.025	5.42E+04	10	5.42E+05	24.45	10.51	11.23	0.99228
		4952.365	3	1650.7883	0.5	20	0.025	6.60E+04	10	6.60E+05	29.78	12.81		
		4028.142	3	1342.714	0.5	20	0.025	5.37E+04	10	5.37E+05	24.22	10.42		
364	TGV112 Unt T2 hr	994.502	3	331.50067	0.5	20	0.025	1.33E+04	10	1.33E+05	5.98	4.76	4.88	0.004
		942.688	3	314.296	0.5	20	0.025	1.26E+04	10	1.26E+05	5.67	4.51		
		1013.606	3	337.86867	0.5	20	0.025	1.35E+04	10	1.35E+05	6.09	4.85		
365	TGV112 5 ug/ml Talc	2450.062	3	816.68733	0.5	20	0.025	3.27E+04	10	3.27E+05	14.73	14.17	14.73	2.7938
		21247.16	3	7082.3867	0.5	20	0.025	2.83E+05	10	2.83E+06	127.76	122.87		
		2644.139	3	881.37967	0.5	20	0.025	3.53E+04	10	3.53E+05	15.90	15.29		
366	TGV112 20 ug/ml Talc	2051.07	3	683.69	0.5	20	0.025	2.73E+04	10	2.73E+05	12.33	11.84	12.13	0.43143
		2061.872	3	687.29067	0.5	20	0.025	2.75E+04	10	2.75E+05	12.40	11.91		
		2191.008	3	730.336	0.5	20	0.025	2.92E+04	10	2.92E+05	13.17	12.65		
367	TGV112 100 ug/ml Talc	7818.098	3	2606.0327	0.5	20	0.025	1.04E+05	10	1.04E+06	47.01	17.38	16.85	1.3486
		7023.288	3	2341.096	0.5	20	0.025	9.36E+04	10	9.36E+05	42.23	15.61		
		7233.577	3	2411.1923	0.5	20	0.025	9.64E+04	10	9.64E+05	43.50	16.08		
368	A3780 Unt T2 hr	833.854	3	277.95133	0.5	20	0.025	1.11E+04	10	1.11E+05	5.01	2.05	2.03	0.2586
		980.02	3	326.67333	0.5	20	0.025	1.31E+04	10	1.31E+05	5.89	2.41		
		907.709	3	302.56967	0.5	20	0.025	1.21E+04	10	1.21E+05	5.46	2.24		
369	5 ug/ml	2907.306	3	969.102	0.5	20	0.025	3.88E+04	10	3.88E+05	17.48	10.21	9.77	0.6736
		2721.779	3	907.25967	0.5	20	0.025	3.63E+04	10	3.63E+05	16.37	9.55		
		2724.242	3	908.08067	0.5	20	0.025	3.63E+04	10	3.63E+05	16.38	9.56		
370	20 ug/ml	3991.927	3	1330.6423	0.5	20	0.025	5.32E+04	10	5.32E+05	24.00	12.02	11.41	0.5468
		3634.487	3	1211.4957	0.5	20	0.025	4.85E+04	10	4.85E+05	21.85	10.94		
		3748.078	3	1249.3593	0.5	20	0.025	5.00E+04	10	5.00E+05	22.54	11.28		
371	100 ug/ml	8451.56	3	2817.1867	0.5	20	0.025	1.13E+05	10	1.13E+06	50.82	20.22	20.74	0.5202
		8687.2	3	2895.7333	0.5	20	0.025	1.16E+05	10	1.16E+06	52.24	20.78		
		8570.22	3	2856.74	0.5	20	0.025	1.18E+05	10	1.18E+06	53.34	21.22		

3/2/2018

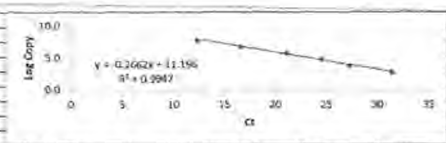
Run PCR – MPO with Samples

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product /Amplicon Length	Start Position
NM_000250	MPO Feb 2	CACTTGTATCCTCTGGTTCTTCATTTATTGAGCACCTACTACATGCAAGGCACTGTACTAGGCGTGAGAAGCATATAGA	CACTTGTATCCTCTGGTTCTT	TCTATATGCTTCTCACGCCT	79	79	2859

Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp	extension time (s) and temp
60	15	63, 60	30, 72

Raw data

Run Summary	(Smart Cycler 2.0d)
Run Name:	MPO 3ul 10x taq
Std Curve:	MPO test stand 60-60 new NK
Started At:	3/2/2018 18:00
Number of Sites:	72

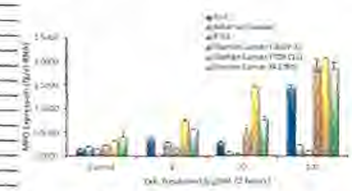


Results Table

Site ID	Protocol	Sample ID	Sample Type	Status	FAM Std/Res	FAM Ct		Cy3 Std/Res	Cy3 Ct	Melt Peak1	Y=Log Copy
B15	60 - 60	7	STD	OK	60900000	12.53		0	17.66	79.47	7.8
B16	60 - 60	6	STD	OK	6090000	16.64		0	20.88	79.2	6.8
C1	60 - 60	5	STD	OK	609000	21.11		0	25.88	79.36	5.8
C2	60 - 60	4	STD	OK	60900	24.45		0	29.26	79.32	4.8
C3	60 - 60	3	STD	OK	6090	27.31		0	31	79.34	3.8
C4	60 - 60	2	STD	OK	609	31.42		0	37.85	79.25	2.8
C5	60 - 60	blank									
A1	MPO - RADIANT SYBR 2017	357	UNKN	OK	645.312	31.50		ND	31.34	79.29	
A2	MPO - RADIANT SYBR 2017		UNKN	OK	416.587	32.22		ND	31.28	79.16	
A3	MPO - RADIANT SYBR 2017		UNKN	OK	745.584	31.27		ND	0	79.24	
A4	MPO - RADIANT SYBR 2017	358	UNKN	OK	591.377	31.55		ND	31.1	79.24	
A5	MPO - RADIANT SYBR 2017		UNKN	OK	552.923	31.76		ND	31.53	79.31	
A6	MPO - RADIANT SYBR 2017		UNKN	OK	525.657	31.84		ND	30.93	79.32	
A7	MPO - RADIANT SYBR 2017	359	UNKN	OK	1796.114	29.83		ND	29.43	78.89	
A8	MPO - RADIANT SYBR 2017		UNKN	OK	2128.677	29.56		ND	29.13	79.21	
A9	MPO - RADIANT SYBR 2017		UNKN	OK	2217.772	29.49		ND	29.44	79.28	
A10	MPO - RADIANT SYBR 2017	361	UNKN	OK	29.258	36.55		ND	35.97	79.16	
A11	MPO - RADIANT SYBR 2017		UNKN	OK	36.743	36.18		ND	36.3	79.28	
A12	MPO - RADIANT SYBR 2017		UNKN	OK	36.982	36.17		ND	36.09	79.13	
A13	MPO - RADIANT SYBR 2017	362	UNKN	OK	82.652	34.86		ND	34.67	79.23	
A14	MPO - RADIANT SYBR 2017		UNKN	OK	103.125	34.50		ND	33.27	79.16	
A15	MPO - RADIANT SYBR 2017		UNKN	OK	107.922	34.42		ND	33.07	79.35	
A16	MPO - RADIANT SYBR 2017	363	UNKN	OK	342.972	32.53		ND	32.25	79.31	
B1	MPO - RADIANT SYBR 2017		UNKN	OK	375.666	32.39		ND	0	79.25	
B2	MPO - RADIANT SYBR 2017		UNKN	OK	214.756	33.30		ND	31.59	79.21	
B3	MPO - RADIANT SYBR 2017	365	UNKN	OK	301.332	32.75		ND	33.86	79.31	
B4	MPO - RADIANT SYBR 2017		UNKN	OK	297.734	32.94		ND	32.93	79.41	
B5	MPO - RADIANT SYBR 2017		UNKN	OK	293.642	32.84		ND	33.12	79.17	
B6	MPO - RADIANT SYBR 2017	366	UNKN	OK	319.869	32.65		ND	32.26	79.18	
B7	MPO - RADIANT SYBR 2017		UNKN	OK	276.118	32.89		ND	32.33	79.17	
B8	MPO - RADIANT SYBR 2017		UNKN	OK	282.458	32.85		ND	33.23	79.36	
B9	MPO - RADIANT SYBR 2017	367	UNKN	OK	948.423	30.88		ND	32.79	79.2	
B10	MPO - RADIANT SYBR 2017		UNKN	OK	912.327	30.94		ND	32.67	79.12	
B11	MPO - RADIANT SYBR 2017		UNKN	OK	924.748	30.92		ND	32.89	79.38	
B12	MPO - RADIANT SYBR 2017	369	UNKN	OK	218.768	33.27		ND	33.21	79.26	
B13	MPO - RADIANT SYBR 2017		UNKN	OK	209.117	33.34		ND	32.98	79.21	
B14	MPO - RADIANT SYBR 2017		UNKN	OK	164.816	33.73		ND	34.54	79.32	
B15	MPO - RADIANT SYBR 2017	370	UNKN	OK	214.533	33.30		ND	32.27	79.14	
B16	MPO - RADIANT SYBR 2017		UNKN	OK	195.546	33.45		ND	33.29	79.41	
C1	MPO - RADIANT SYBR 2017		UNKN	OK	212.351	33.32		ND	32.63	79.28	
C2	MPO - RADIANT SYBR 2017	371	UNKN	OK	805.293	31.14		ND	30.86	79.42	
C3	MPO - RADIANT SYBR 2017		UNKN	OK	706.822	31.35		ND	30.85	79.23	
C4	MPO - RADIANT SYBR 2017		UNKN	OK	853.694	31.05		ND	30.41	79.29	
C5	MPO - RADIANT SYBR 2017	379	UNKN	OK	669.3558	36.41		ND	31.09	79.3	
C6	MPO - RADIANT SYBR 2017		UNKN	OK	664.4529	36.57		ND	30.96	79.21	
C7	MPO - RADIANT SYBR 2017		UNKN	OK	669.0931	36.60		ND	30.87	79.12	
A1	MPO - RADIANT SYBR 2017	380	UNKN	OK	551.88	34.64		ND	28.49	78.82	
A2	MPO - RADIANT SYBR 2017		UNKN	OK	327.683	34.69		ND	27.55	78.84	
A3	MPO - RADIANT SYBR 2017		UNKN	OK	231.01065	34.67		ND	0	78.73	
A4	MPO - RADIANT SYBR 2017	381	UNKN	OK	12860.656	37.03		ND	27.51	78.77	
A5	MPO - RADIANT SYBR 2017		UNKN	OK	11507.04	37.16		ND	28.56	78.53	
A6	MPO - RADIANT SYBR 2017		UNKN	OK	10002.198	37.43		ND	28.15	78.7	
A7	MPO - RADIANT SYBR 2017	382	UNKN	OK	454.313	37.05		ND	33.23	79.04	
A8	MPO - RADIANT SYBR 2017		UNKN	OK	434.4	37.21		ND	33.1	78.96	
A9	MPO - RADIANT SYBR 2017		UNKN	OK	605.783	37.06		ND	33.12	78.78	
A10	MPO - RADIANT SYBR 2017	383	UNKN	OK	459.976	36.07		ND	33.23	78.91	
A11	MPO - RADIANT SYBR 2017		UNKN	OK	270.276	36.18		ND	33.89	79.04	
A12	MPO - RADIANT SYBR 2017		UNKN	OK	335.145	36.26		ND	33.58	79.21	
A13	MPO - RADIANT SYBR 2017	384	UNKN	OK	1506.613	38.20		ND	31.92	78.97	
A14	MPO - RADIANT SYBR 2017		UNKN	OK	1446.257	38.28		ND	30.57	78.91	
A15	MPO - RADIANT SYBR 2017		UNKN	OK	1187.594	38.16		ND	30.59	78.86	
A16	MPO - RADIANT SYBR 2017	385	UNKN	OK	1162.386	37.64		ND	31.26	79.02	
B1	MPO - RADIANT SYBR 2017		UNKN	OK	886.57	37.37		ND	0	79.08	
B2	MPO - RADIANT SYBR 2017		UNKN	OK	755.896	37.67		ND	31.5	79.12	
B3	MPO - RADIANT SYBR 2017	386	UNKN	OK	855.386	35.67		ND	33.71	78.97	
B4	MPO - RADIANT SYBR 2017		UNKN	OK	981.45	35.79		ND	32.26	79.13	
B5	MPO - RADIANT SYBR 2017		UNKN	OK	1212.295	35.72		ND	31.88	79.18	

Calculation

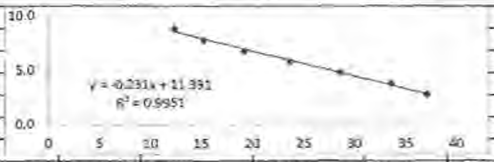
Gene of I-A1:O86nterest	MPO		
		Unit	Formula
1 Dalton = 1.66E-24 grams	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire gene	79	bases	
Mass in Daltons	2.41E+04	Da	= number bases x avg. mass/base
Mass in grams	4.00E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	4.00E-14	ug	= above / 10E-6
Mass in ng	4.00E-11	ng/copy	= above x 10E3
3/2/2018 18:00			



ID	Sample	Copy #	ul cDNA used	copies/ul cDNA	ug RNA used	ul cDNA made	ug RNA/ul cDNA	copies/ug RNA	Dilution Factor	copies/ul RNA x DF	fg/ul RNA	Normalized	Average	SD
357	EL1 5 ug/ml Talc	645.312	3	215.104	0.5	20	0.025	8.60E+03	10	8.60E+04	3.44E+00	3.38	0.3617	0.0389
		416.587	3	138.86233	0.5	20	0.025	5.55E+03	10	5.55E+04	2.22E+00	2.17		
		745.584	3	248.528	0.5	20	0.025	9.94E+03	10	9.94E+04	3.98E+00	3.88		
358	EL1 20 ug/ml Talc	591.377	3	197.12567	0.5	20	0.025	7.89E+03	10	7.89E+04	3.16E+00	3.10	0.2926	0.0173
		552.923	3	184.30767	0.5	20	0.025	7.37E+03	10	7.37E+04	2.95E+00	2.90		
		525.657	3	175.219	0.5	20	0.025	7.01E+03	10	7.01E+04	2.81E+00	2.75		
359	EL1 100 ug/ml Talc	1796.114	3	598.70467	0.5	20	0.025	2.39E+04	10	2.39E+05	9.58E+00	12.03	1.4564	0.0422
		2128.677	3	709.559	0.5	20	0.025	2.84E+04	10	2.84E+05	1.14E+01	14.26		
		2217.772	3	739.25733	0.5	20	0.025	2.96E+04	10	2.96E+05	1.18E+01	14.85		
383	Normal Ovarian Unt 72 hr	39.259	3	13.086	0.5	20	0.025	5.23E+02	10	5.23E+03	2.10E-01	0.21	0.1974	0.0116
		36.743	3	12.247667	0.5	20	0.025	4.90E+02	10	4.90E+03	1.96E-01	0.20		
		34.932	3	11.644	0.5	20	0.025	4.66E+02	10	4.66E+03	1.86E-01	0.19		
384	5 ug/ml	10.652	3	3.5506667	0.5	20	0.025	1.42E+02	10	1.42E+03	5.69E-02	0.05	0.0533	0.0021
		10.123	3	3.3743333	0.5	20	0.025	1.35E+02	10	1.35E+03	5.40E-02	0.05		
		10.932	3	3.644	0.5	20	0.025	1.46E+02	10	1.46E+03	5.83E-02	0.06		
385	20 ug/ml	14.972	3	4.9906667	0.5	20	0.025	2.00E+02	10	2.00E+03	7.99E-02	0.08	0.0853	0.0062
		17.666	3	5.8886667	0.5	20	0.025	2.36E+02	10	2.36E+03	9.43E-02	0.09		
		14.756	3	4.9186667	0.5	20	0.025	1.97E+02	10	1.97E+03	7.88E-02	0.08		
386	100 ug/ml	50.332	3	16.777333	0.5	20	0.025	6.71E+02	10	6.71E+03	2.69E-01	0.24	0.2336	0.0087
		46.734	3	15.578	0.5	20	0.025	6.23E+02	10	6.23E+03	2.49E-01	0.22		
		48.642	3	16.214	0.5	20	0.025	6.48E+02	10	6.48E+03	2.60E-01	0.23		
379	FT33 Unt 72 hr	31.869	3	10.623	0.5	20	0.025	4.25E+02	10	4.25E+03	1.70E-01	0.17	0.1483	0.0051
		27.118	3	9.0393333	0.5	20	0.025	3.62E+02	10	3.62E+03	1.45E-01	0.14		
		28.458	3	9.486	0.5	20	0.025	3.79E+02	10	3.79E+03	1.52E-01	0.15		
380	5 ug/ml Talc	94.423	3	31.474333	0.5	20	0.025	1.26E+03	10	1.26E+04	5.04E-01	0.30	0.2927	0.0049
		91.327	3	30.442333	0.5	20	0.025	1.22E+03	10	1.22E+04	4.87E-01	0.29		
		92.748	3	30.916	0.5	20	0.025	1.24E+03	10	1.24E+04	4.95E-01	0.29		
381	20 ug/ml Talc	21.768	3	7.256	0.5	20	0.025	2.90E+02	10	2.90E+03	1.16E-01	0.06	0.0514	0.0072
		20.117	3	6.7056667	0.5	20	0.025	2.68E+02	10	2.68E+03	1.07E-01	0.05		
		16.444	3	5.4813333	0.5	20	0.025	2.19E+02	10	2.19E+03	8.78E-02	0.04		
382	100 ug/ml Talc	21.533	3	7.1776667	0.5	20	0.025	2.87E+02	10	2.87E+03	1.15E-01	0.11	0.1111	0.0058
		19.562	3	6.5206667	0.5	20	0.025	2.61E+02	10	2.61E+03	1.04E-01	0.10		
		21.351	3	7.117	0.5	20	0.025	2.85E+02	10	2.85E+03	1.14E-01	0.11		
361	SKOV 5 ug/ml	29.258	3	9.7526667	0.5	20	0.025	3.90E+02	10	3.90E+03	1.56E-01	0.15	0.1745	0.0223
		36.743	3	12.247667	0.5	20	0.025	4.90E+02	10	4.90E+03	1.96E-01	0.19		
		36.982	3	12.327333	0.5	20	0.025	4.93E+02	10	4.93E+03	1.97E-01	0.19		
362	20 ug/ml	82.652	3	27.550667	0.5	20	0.025	1.10E+03	10	1.10E+04	4.41E-01	0.40	0.5075	0.0645
		103.125	3	34.375	0.5	20	0.025	1.38E+03	10	1.38E+04	5.50E-01	0.50		
		107.922	3	35.974	0.5	20	0.025	1.44E+03	10	1.44E+04	5.76E-01	0.52		
363	100 ug/ml	342.972	3	114.324	0.5	20	0.025	4.57E+03	10	4.57E+04	1.83E+00	1.83	1.0178	0.1234
		375.666	3	125.222	0.5	20	0.025	5.01E+03	10	5.01E+04	2.01E+00	2.01		
		214.756	3	71.585333	0.5	20	0.025	2.86E+03	10	2.86E+04	1.15E+00	1.15		
365	TOV112 5 ug/ml Talc	301.332	3	100.444	0.5	20	0.025	4.02E+03	10	4.02E+04	1.61E+00	0.80	0.7513	0.0444
		267.734	3	89.244667	0.5	20	0.025	3.57E+03	10	3.57E+04	1.43E+00	0.71		
		283.642	3	94.547333	0.5	20	0.025	3.78E+03	10	3.78E+04	1.51E+00	0.75		
366	TOV112 20 ug/ml Talc	319.869	3	106.623	0.5	20	0.025	4.26E+03	10	4.26E+04	1.71E+00	1.71	1.4907	0.0239
		276.118	3	92.039333	0.5	20	0.025	3.68E+03	10	3.68E+04	1.47E+00	1.47		
		282.458	3	94.152667	0.5	20	0.025	3.77E+03	10	3.77E+04	1.51E+00	1.51		
367	TOV112 100 ug/ml Talc	948.423	3	316.141	0.5	20	0.025	1.26E+04	10	1.26E+05	5.06E+00	2.12	2.0731	0.0409
		912.327	3	304.109	0.5	20	0.025	1.22E+04	10	1.22E+05	4.87E+00	2.04		
		924.748	3	308.24933	0.5	20	0.025	1.23E+04	10	1.23E+05	4.94E+00	2.06		
369	A2780 5 ug/ml	218.768	3	72.922667	0.5	20	0.025	2.92E+03	10	2.92E+04	1.17E+00	0.58	0.5645	0.0120
		209.117	3	69.705667	0.5	20	0.025	2.79E+03	10	2.79E+04	1.12E+00	0.55		
		164.816	3	54.938667	0.5	20	0.025	2.20E+03	10	2.20E+04	8.80E-01	0.43		
370	20 ug/ml	214.533	3	71.511	0.5	20	0.025	2.86E+03	10	2.86E+04	1.15E+00	0.82	0.7924	0.0337
		195.546	3	65.182	0.5	20	0.025	2.61E+03	10	2.61E+04	1.04E+00	0.75		
		212.351	3	70.783667	0.5	20	0.025	2.83E+03	10	2.83E+04	1.13E+00	0.81		
371	100 ug/ml	805.293	3	268.431	0.5	20	0.025	1.07E+04	10	1.07E+05	4.30E+00	1.85	1.9039	0.0786
		706.822	3	235.60733	0.5	20	0.025	9.42E+03	10	9.42E+04	3.77E+00	1.62		
		853.694	3	284.56467	0.5	20	0.025	1.14E+04	10	1.14E+05	4.56E+00	1.96		
355	EL1 unt	669.355	3	223.11833	0.5	20	0.025	8.92E+03	10	8.92E+04	3.57E+00	1.46	0.1480	0.0060
		664.453	3	221.48433	0.5	20	0.025	8.86E+03	10	8.86E+04	3.55E+00	1.45		
		669.093	3	223.031	0.5	20	0.025	8.92E+03	10	8.92E+04	3.57E+00	1.46		
360	SKOV-3 unt	454.313	3	151.43767	0.5	20	0.025	6.06E+03	10	6.06E+04	2.42E+00	0.23	0.2242	0.0071
		434.4	3	144.8	0.5	20	0.025	5.79E+03	10	5.79E+04	2.32E+00	0.22		
		605.783	3	201.92767	0.5	20	0.025	8.08E+03	10	8.08E+04	3.23E+00	0.31		
368	A2780 unt	1162.386	3	387.462	0.5	20	0.025	1.55E+04	10	1.55E+05	6.20E+00	0.82	0.4364	0.0494
		886.67	3	295.55667	0.5	20	0.025	1.18E+04	10	1.18E+05	4.73E+00	0.47		
		755.896	3	251.96533	0.5	20	0.025	1.01E+04	10	1.01E+05	4.03E+00	0.40		
364	TOV112 unt	1305.857	3	435.28567	0.5	20	0.025	1.74E+04	10	1.74E+05	6.97E+00	0.41	0.3211	0.0141
		1049.829	3	349.943	0.5	20	0.025	1.40E+04	10	1.40E+05	5.60E+00	0.33		
		986.582	3	328.86067	0.5	20	0.025	1.32E+04	10	1.32E+05	5.27E+00	0.31		

3/2/2018
Run PCR – GPX samples

Run Summary	(Smart Cycler 2.0d)
Run Name:	GPX Talc
Std Curve:	GPX Standard RADIANT
Started At:	3/2/2018 14:44
Number of :	72



Results Table

Site ID	Protocol	Sample ID		Sample Typ	Status	FAM Std/Res	FAM Ct	Melt Peak1
A1	GSTp1 - RADIANT SYBR 201		8	STD	OK	608000000	12.29	82.73
A2	GSTp1 - RADIANT SYBR 201		7	STD	OK	60800000	13.15	82.77
A3	GSTp1 - RADIANT SYBR 201		6	STD	OK	6080000	16.12	82.76
A4	GSTp1 - RADIANT SYBR 201		5	STD	OK	608000	20.69	82.3
A5	GSTp1 - RADIANT SYBR 201		4	STD	OK	60800	24.74	82.72
A6	GSTp1 - RADIANT SYBR 201		3	STD	OK	6080	28.15	82.87
A7	GSTp1 - RADIANT SYBR 201		2	STD	OK	608	31.71	82.84
B1	GSTp1 - RADIANT SYBR 201		357	UNKN	OK	668201.923	20.84	82.11
B2	GSTp1 - RADIANT SYBR 2018			UNKN	OK	666753.61	20.84	82.95
B3	GSTp1 - RADIANT SYBR 2018			UNKN	OK	671705.856	20.83	82.07
B4	GSTp1 - RADIANT SYBR 201		358	UNKN	OK	206839.922	22.63	82.88
B5	GSTp1 - RADIANT SYBR 2018			UNKN	OK	230366.035	22.46	82.87
B6	GSTp1 - RADIANT SYBR 2018			UNKN	OK	210731.99	22.60	82.1
B7	GSTp1 - RADIANT SYBR 201		359	UNKN	OK	64785.937	24.40	82.1
B8	GSTp1 - RADIANT SYBR 2018			UNKN	OK	65867.594	24.38	82.75
B9	GSTp1 - RADIANT SYBR 2018			UNKN	OK	65675.403	24.38	82
B10	GSTp1 - RADIANT SYBR 201		361	UNKN	OK	508032.78	21.25	82
B11	GSTp1 - RADIANT SYBR 2018			UNKN	OK	479704.249	21.34	82.16
B12	GSTp1 - RADIANT SYBR 2018			UNKN	OK	498208.949	21.32	82.97
B13	GSTp1 - RADIANT SYBR 201		362	UNKN	OK	277671.948	22.18	82.91
B14	GSTp1 - RADIANT SYBR 2018			UNKN	OK	285591.813	22.14	82.03
B15	GSTp1 - RADIANT SYBR 2018			UNKN	OK	257832.145	22.29	82.9
B16	GSTp1 - RADIANT SYBR 201		363	UNKN	OK	195790.778	22.71	82.06
A1	GSTp1 - RADIANT SYBR 2018			UNKN	OK	174633.209	22.89	82.13
A2	GSTp1 - RADIANT SYBR 2018			UNKN	OK	193958.071	22.73	82.87
A3	GSTp1 - RADIANT SYBR 201		365	UNKN	OK	382147.473	21.69	82.17
A4	GSTp1 - RADIANT SYBR 2018			UNKN	OK	382528.579	21.69	82.13
A5	GSTp1 - RADIANT SYBR 2018			UNKN	OK	381507.876	21.69	82.15
A6	GSTp1 - RADIANT SYBR 201		366	UNKN	OK	165461.759	22.97	82.02
A7	GSTp1 - RADIANT SYBR 2018			UNKN	OK	142225.778	23.20	82.06
A8	GSTp1 - RADIANT SYBR 2018			UNKN	OK	148912.529	23.13	82.1
A9	GSTp1 - RADIANT SYBR 201		367	UNKN	OK	199402.777	22.68	82.04
A10	GSTp1 - RADIANT SYBR 2018			UNKN	OK	128707.691	23.35	82.91
A11	GSTp1 - RADIANT SYBR 2018			UNKN	OK	187371.231	22.76	82.29
A12	GSTp1 - RADIANT SYBR 201		369	UNKN	OK	454082.582	21.43	82.02
A13	GSTp1 - RADIANT SYBR 2018			UNKN	OK	411760.96	21.58	82.2
A14	GSTp1 - RADIANT SYBR 2018			UNKN	OK	439283.754	21.48	82.14
A15	GSTp1 - RADIANT SYBR 201		370	UNKN	OK	185507.125	22.80	82.17
A16	GSTp1 - RADIANT SYBR 2018			UNKN	OK	199908.926	22.68	82.1
C1	GSTp1 - RADIANT SYBR 2018			UNKN	OK	150814.91	23.11	82.19
C2	GSTp1 - RADIANT SYBR 201		371	UNKN	OK	90033.388	23.90	82.12
C3	GSTp1 - RADIANT SYBR 2018			UNKN	OK	92582.039	23.86	82.19
C4	GSTp1 - RADIANT SYBR 2018			UNKN	OK	77597.643	24.13	82.01
C5	GSTp1 - RADIANT SYBR 201		379	UNKN	OK	812750.693	22.97	82.21
C6	GSTp1 - RADIANT SYBR 2018			UNKN	OK	803430.814	23.20	82.21
C7	GSTp1 - RADIANT SYBR 2018			UNKN	OK	832511.564	23.13	82.79
B1	GSTp1 - RADIANT SYBR 201		380	UNKN	OK	600265.246	22.06	82.09
B2	GSTp1 - RADIANT SYBR 2018			UNKN	OK	562024.568	22.48	82.88
B3	GSTp1 - RADIANT SYBR 2018			UNKN	OK	1175903.995	22.13	82.04
B4	GSTp1 - RADIANT SYBR 201		381	UNKN	OK	718719.203	22.73	82.99
B5	GSTp1 - RADIANT SYBR 2018			UNKN	OK	680134.125	22.59	82.91
B6	GSTp1 - RADIANT SYBR 2018			UNKN	OK	572473.147	22.76	82.96
B7	GSTp1 - RADIANT SYBR 201		382	UNKN	OK	105507.125	23.66	82.98
B8	GSTp1 - RADIANT SYBR 2018			UNKN	OK	109908.926	23.60	82.55
B9	GSTp1 - RADIANT SYBR 2018			UNKN	OK	90980.998	23.89	82.94
B10	GSTp1 - RADIANT SYBR 201		383	UNKN	OK	1051419.196	21.25	82.98
B11	GSTp1 - RADIANT SYBR 2018			UNKN	OK	836587.722	21.34	82.03
B12	GSTp1 - RADIANT SYBR 2018			UNKN	OK	945596.546	21.32	82.02
B13	GSTp1 - RADIANT SYBR 201		384	UNKN	OK	800078.097	21.71	82.94
B14	GSTp1 - RADIANT SYBR 2018			UNKN	OK	851661.834	21.68	82.9
B15	GSTp1 - RADIANT SYBR 2018			UNKN	OK	706709.075	21.79	82.97
B16	GSTp1 - RADIANT SYBR 201		385	UNKN	OK	503377.481	22.97	82.17
A1	GSTp1 - RADIANT SYBR 2018			UNKN	OK	291290.52	22.89	82.15
A2	GSTp1 - RADIANT SYBR 2018			UNKN	OK	221345.011	22.73	82.95
A3	GSTp1 - RADIANT SYBR 201		386	UNKN	OK	333584.129	23.31	82.06
A4	GSTp1 - RADIANT SYBR 2018			UNKN	OK	344465.658	23.31	82.19
A5	GSTp1 - RADIANT SYBR 2018			UNKN	OK	348884.247	23.32	82.14
A7	GSTp1 - RADIANT SYBR 202		364	UNKN	OK	238896.626	22.41	82.2
A8	GSTp1 - RADIANT SYBR 2021			UNKN	OK	189277.851	22.76	82.09
A9	GSTp1 - RADIANT SYBR 2022			UNKN	OK	246310.592	22.36	83.18
A10	GSTp1 - RADIANT SYBR 202		360	UNKN	OK	981714.39	20.25	82.16
A11	GSTp1 - RADIANT SYBR 2024			UNKN	OK	970207.832	20.26	82.09
A12	GSTp1 - RADIANT SYBR 2025			UNKN	OK	1098811.105	20.07	83.34
A13	GSTp1 - RADIANT SYBR 202		356	UNKN	OK	812750.693	20.54	82.09
A14	GSTp1 - RADIANT SYBR 2027			UNKN	OK	803430.814	20.55	82.52
A15	GSTp1 - RADIANT SYBR 2028			UNKN	OK	832511.564	20.50	82.11
A16	GSTp1 - RADIANT SYBR 202		368	UNKN	OK	503377.481	21.27	82.91
A17	GSTp1 - RADIANT SYBR 2030			UNKN	OK	291290.52	22.11	82.12
A18	GSTp1 - RADIANT SYBR 2031			UNKN	OK	221345.011	22.53	83.12

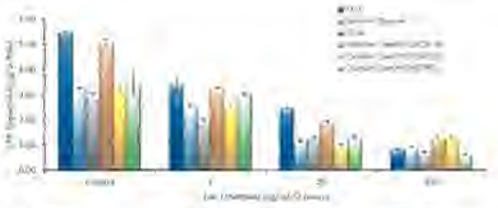
UB

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product /Amplicon Length	Start Position
NM_000581	GPX	GGACTACACCCAGATGAACGAGCTGCAGCGGCGCCT CGGACCCCGGGGCCTGGTGGTGCTCGGCTTCCCGTG CAACCAGTTTGGGCATCAGGAGAA	GGACTACACCCAGATGAAC	TTCTCCTGATGCCCAAAC	100	100	242

Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp	extension time (s) and temp
60	15	10, 60	30, 72

Primer information & calculation

Gene of Interest	GPX					
		Units	Formula			
1 Dalton = 1.66E-24 grams	1.66E-24	g				
Mass of base pair	615	Da				
Avg. Mass/base	305.25	Da				
Length of entire gene	1,004	bases				
Mass in Daltons	3.05E+04	Da	= number bases x avg. mass/base			
Mass in grams	5.07E-20	g	= mass in Da x mass of a Da in grams			
Mass in ug	5.07E-14	ug	= above / 10E-6			
Mass in ng	5.07E-11	ng/copy	= above x 10E3			
ID	Sample	Copy #	ul cDNA used	copies/ul cDNA		



GSTp1 - RADIANT SYBR 2018													
ID	Sample	Copy #	ul cDNA used	copies/ul cDNA	ug RNA used	ul cDNA made	ug RNA/ul cDNA	copies/ug RNA	Dilution Factor	copies/ul cDNA x DF	pg/ul RNA	Normalized	Average	SD
357	EL1 5 ug/ml Talc	468201.923	3	156067.308	0.5	20	0.025	6.24E+06	10	6.24E+07	3.10E+00	3.08	3.42	0.34
		366753.61	3	122251.203	0.5	20	0.025	4.89E+06	10	4.89E+07	2.48E+00	2.41		
		571705.856	3	190568.619	0.5	20	0.025	7.62E+06	10	7.62E+07	3.86E+00	3.76		
358	EL1 20 ug/ml Talc	326839.922	3	108946.641	0.5	20	0.025	4.36E+06	10	4.36E+07	2.21E+00	2.17	2.47	0.05
		370366.035	3	123455.345	0.5	20	0.025	4.94E+06	10	4.94E+07	2.50E+00	2.46		
		360731.99	3	120243.997	0.5	20	0.025	4.81E+06	10	4.81E+07	2.44E+00	2.39		
359	EL1 100 ug/ml Talc	114785.937	3	38261.979	0.5	20	0.025	1.53E+06	10	1.53E+07	7.76E-01	0.97	0.81	0.05
		125867.594	3	41955.8647	0.5	20	0.025	1.68E+06	10	1.68E+07	8.50E-01	1.07		
		195675.403	3	65225.1343	0.5	20	0.025	2.61E+06	10	2.61E+07	1.32E+00	1.66		
383	Normal Ovarian lot# 72 hr	508032.76	3	169344.26	0.5	20	0.025	6.77E+06	10	6.77E+07	3.43E+00	3.43	3.27	0.04
		479704.249	3	159901.416	0.5	20	0.025	6.40E+06	10	6.40E+07	3.24E+00	3.24		
		488208.949	3	162736.316	0.5	20	0.025	6.51E+06	10	6.51E+07	3.30E+00	3.30		
384	5 ug/ml	377671.948	3	125890.649	0.5	20	0.025	5.04E+06	10	5.04E+07	2.55E+00	2.41	2.58	0.04
		385591.813	3	128530.604	0.5	20	0.025	5.14E+06	10	5.14E+07	2.61E+00	2.48		
		357832.145	3	119277.382	0.5	20	0.025	4.77E+06	10	4.77E+07	2.42E+00	2.29		
385	20 ug/ml	195790.778	3	65263.5927	0.5	20	0.025	2.61E+06	10	2.61E+07	1.32E+00	1.07	1.15	0.04
		174633.209	3	58211.0697	0.5	20	0.025	2.33E+06	10	2.33E+07	1.18E+00	1.12		
		193958.071	3	64652.6903	0.5	20	0.025	2.59E+06	10	2.59E+07	1.31E+00	1.25		
386	100 ug/ml	132147.473	3	44049.1577	0.5	20	0.025	1.76E+06	10	1.76E+07	8.93E-01	0.80	0.89	0.00
		132528.579	3	44176.193	0.5	20	0.025	1.77E+06	10	1.77E+07	8.98E-01	0.81		
		131507.876	3	43835.9587	0.5	20	0.025	1.75E+06	10	1.75E+07	8.86E-01	0.80		
379	FT33 lot# 72 hr	465461.759	3	155153.92	0.5	20	0.025	6.21E+06	10	6.21E+07	3.14E+00	3.14	3.01	0.03
		442225.778	3	147408.593	0.5	20	0.025	5.90E+06	10	5.90E+07	2.99E+00	2.99		
		448812.529	3	149604.176	0.5	20	0.025	5.98E+06	10	5.98E+07	3.03E+00	3.03		
380	5 ug/ml Talc	299402.777	3	99800.9257	0.5	20	0.025	3.99E+06	10	3.99E+07	2.02E+00	1.20	1.08	0.06
		228707.691	3	76235.897	0.5	20	0.025	3.05E+06	10	3.05E+07	1.55E+00	0.91		
		287371.231	3	95790.4103	0.5	20	0.025	3.83E+06	10	3.83E+07	1.94E+00	1.15		
381	20 ug/ml Talc	194082.582	3	64694.194	0.5	20	0.025	2.59E+06	10	2.59E+07	1.31E+00	0.65	1.30	0.02
		211760.96	3	70586.9867	0.5	20	0.025	2.82E+06	10	2.82E+07	1.43E+00	0.71		
		189283.754	3	63094.5847	0.5	20	0.025	2.52E+06	10	2.52E+07	1.28E+00	0.63		
382	100 ug/ml Talc	105507.125	3	35169.0417	0.5	20	0.025	1.41E+06	10	1.41E+07	7.13E-01	0.71	0.71	0.02
		109908.926	3	36636.3087	0.5	20	0.025	1.47E+06	10	1.47E+07	7.43E-01	0.74		
		90980.998	3	30326.9993	0.5	20	0.025	1.21E+06	11	1.33E+07	6.76E-01	0.68		
381	SKOV 5 ug/ml	508032.78	3	169344.26	0.5	20	0.025	6.77E+06	10	6.77E+07	3.43E+00	1.44	1.37	0.04
		479704.249	3	159901.416	0.5	20	0.025	6.40E+06	10	6.40E+07	3.24E+00	1.36		
		488208.949	3	162736.316	0.5	20	0.025	6.51E+06	10	6.51E+07	3.30E+00	1.38		
382	20 ug/ml	277671.948	3	92557.316	0.5	20	0.025	3.70E+06	10	3.70E+07	1.88E+00	1.88	1.90	0.04
		285591.813	3	95197.271	0.5	20	0.025	3.81E+06	10	3.81E+07	1.93E+00	1.93		
		257832.145	3	85944.0483	0.5	20	0.025	3.44E+06	10	3.44E+07	1.74E+00	1.74		
383	100 ug/ml	195790.778	3	65263.5927	0.5	20	0.025	2.61E+06	10	2.61E+07	1.32E+00	0.85	1.25	0.10
		174633.209	3	58211.0697	0.5	20	0.025	2.33E+06	10	2.33E+07	1.18E+00	0.58		
		193958.071	3	64652.6903	0.5	20	0.025	2.59E+06	10	2.59E+07	1.31E+00	0.65		
385	TOV112 5 ug/ml Talc	382147.473	3	127382.491	0.5	20	0.025	5.10E+06	10	5.10E+07	2.58E+00	1.65	2.58	0.00
		382528.579	3	127509.526	0.5	20	0.025	5.10E+06	10	5.10E+07	2.58E+00	1.65		
		381507.876	3	127169.292	0.5	20	0.025	5.09E+06	10	5.09E+07	2.58E+00	1.64		
368	TOV112 20 ug/ml Talc	165461.759	3	55153.9197	0.5	20	0.025	2.21E+06	10	2.21E+07	1.12E+00	0.48	0.90	0.03
		142225.778	3	47408.5927	0.5	20	0.025	1.90E+06	10	1.90E+07	9.61E-01	0.41		
		148812.529	3	49604.1763	0.5	20	0.025	1.98E+06	10	1.98E+07	1.01E+00	0.43		
367	TOV112 100 ug/ml Talc	199402.777	3	66467.5923	0.5	20	0.025	2.66E+06	10	2.66E+07	1.35E+00	1.07	1.31	0.06
		128707.691	3	42902.5637	0.5	20	0.025	1.72E+06	10	1.72E+07	8.70E-01	0.69		
		187371.231	3	62457.077	0.5	20	0.025	2.50E+06	10	2.50E+07	1.27E+00	1.01		
369	A2780 5 ug/ml	454082.582	3	151360.861	0.5	20	0.025	6.05E+06	10	6.05E+07	3.07E+00	2.85	3.02	0.07
		411760.96	3	137253.653	0.5	20	0.025	5.49E+06	10	5.49E+07	2.78E+00	2.68		
		439263.754	3	146427.918	0.5	20	0.025	5.86E+06	10	5.86E+07	2.97E+00	2.85		
370	20 ug/ml	185507.125	3	61835.7083	0.5	20	0.025	2.47E+06	10	2.47E+07	1.25E+00	1.20	1.30	0.07
		199908.926	3	66636.3087	0.5	20	0.025	2.67E+06	10	2.67E+07	1.35E+00	1.30		
		150814.91	3	50271.6367	0.5	20	0.025	2.01E+06	10	2.01E+07	1.02E+00	0.98		
371	100 ug/ml	90033.389	3	30011.1293	0.5	20	0.025	1.20E+06	10	1.20E+07	6.08E-01	0.22	0.62	0.01
		92582.039	3	30860.8797	0.5	20	0.025	1.23E+06	10	1.23E+07	6.20E-01	0.23		
		77597.643	3	25865.881	0.5	20	0.025	1.03E+06	10	1.03E+07	5.24E-01	0.19		
368	EL1 unt	812750.693	3	270916.898	0.5	20	0.025	1.08E+07	10	1.08E+08	5.49E+00	5.35	5.48	0.04
		803430.814	3	267810.271	0.5	20	0.025	1.07E+07	10	1.07E+08	5.43E+00	5.29		
		832511.564	3	277503.855	0.5	20	0.025	1.11E+07	10	1.11E+08	5.62E+00	5.48		
380	SKOV-3 unt	381714.39	3	127238.13	0.5	20	0.025	5.09E+06	20	1.02E+08	5.15E+00	4.87	5.08	0.11
		370207.832	3	123402.611	0.5	20	0.025	4.94E+06	20	9.87E+07	5.00E+00	4.73		
		398611.105	3	132937.035	0.5	20	0.025	5.32E+06	20	1.06E+08	5.39E+00	5.09		
388	A2780 unt	503377.481	3	167792.494	0.5	20	0.025	6.71E+06	20	1.34E+08	6.80E+00	6.66	6.60	0.47
		291290.52	3	97096.84	0.5	20	0.025	3.88E+06	20	7.77E+07	3.94E+00	3.94		
		221345.011	3	73781.6703	0.5	20	0.025	2.95E+06	20	5.90E+07	2.99E+00	2.89		
384	TOV112 unt	238896.626	3	79632.2087	0.5	20	0.025	3.19E+06	20	6.37E+07	3.23E+00	1.91	3.28	0.07
		189277.851	3	63092.617	0.5	20	0.025	2.52E+06	20	5.05E+07	2.56E+00	1.51		
		246310.592	3	82103.5307	0.5	20	0.025	3.28E+06	20	6.57E+07	3.33E+00	1.97		

SAED000022(color)

3/2/2018

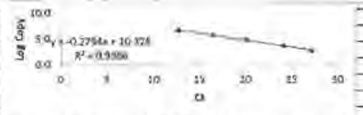
Run PCR - SOD3 with Samples 356 ~ 386

Accession #	Gene	Sequence	Fwd Primer	Rev Primer	Standard Length	Product /Amplicon Length
NM_000636	SOD3	GCGGTAGCACCAGCACTAGCAGCATGTTGAGCCGGG CAGTGTGCGGCACCAGCAGGCAGCTGGCTCCGGTTT TGGGGTATCTGGGCTCC	GCGGTAGCACCAGCACTA	GGAGCCCAGATACCCCAA	85	85

Primer information

Start Position	Initial time (s) at 95 C	Melt time at 95 C	Anneal time (s) and Temp	extension time (s) and temp
132	60	15	10, 60	30, 72

Run Summary	(Smart Cycler 2.0d)
Run Name:	SOD 3ul 10x tolc
Std Curve:	SOD test stand 60-60 new NK
Started At:	3/2/2018 18:00
Number of Sites:	72



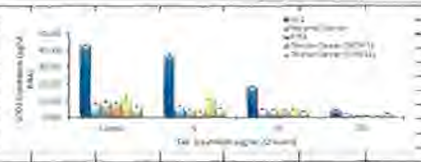
Results Table

Site ID	Protocol	Sample ID	Sample Type	Status	FAM Std/Res	FAM Ct	Cy3 Std/Res	Cy3 Ct	Melt Peak1	log copies
B15	60 - 60	8	STD	OK	610000000	0	0	0	86.47	
B16	60 - 60	7	STD	OK	67000000	0	0	0	86.2	
C1	60 - 60	6	STD	OK	6999999.5	12.75	0	16.88	86.36	
C2	60 - 60	5	STD	OK	610000	16.52	0	20.46	86.37	
C3	60 - 60	4	STD	OK	61000	20.15	0	23.88	86.32	
C4	60 - 60	3	STD	OK	6100	24.06	0	27.82	86.34	
C5	60 - 60	2	STD	OK	610	27.11	0	30.8	85.86	
	SOD - RADIANT SYBR 2017	356	UNKN	OK	7893.555	23.58	0	31.34	85.98	
	SOD - RADIANT SYBR 2018		UNKN	OK	7644.539	23.39	0	31.34	85.98	
	SOD - RADIANT SYBR 2019		UNKN	OK	7690.931	23.25	0	31.34	85.96	
A1	SOD - RADIANT SYBR 2020	357	UNKN	OK	8645.212	21.61	ND	31.34	86.25	
A2	SOD - RADIANT SYBR 2017		UNKN	OK	8416.587	23.67	ND	31.28	85.99	
A3	SOD - RADIANT SYBR 2017		UNKN	OK	6745.534	23.59	ND	0	86.16	
A4	SOD - RADIANT SYBR 2017	358	UNKN	OK	2691.377	25.10	ND	31.1	86.24	
A5	SOD - RADIANT SYBR 2017		UNKN	OK	2552.923	25.13	ND	31.53	86.1	
A6	SOD - RADIANT SYBR 2017		UNKN	OK	2525.657	25.14	ND	30.58	86.31	
A7	SOD - RADIANT SYBR 2017	359	UNKN	OK	796.114	26.96	ND	29.43	86.32	
A8	SOD - RADIANT SYBR 2017		UNKN	OK	728.671	27.10	ND	29.13	85.84	
A9	SOD - RADIANT SYBR 2017		UNKN	OK	717.772	27.12	ND	29.44	86.26	
A10	SOD - RADIANT SYBR 2018	360	UNKN	OK	1454.313	26.01	ND	30.44	86.29	
A11	SOD - RADIANT SYBR 2019		UNKN	OK	1434.403	26.03	ND	31.44	86.26	
A12	SOD - RADIANT SYBR 2020		UNKN	OK	1605.783	25.85	ND	32.44	86.26	
A10	SOD - RADIANT SYBR 2017	361	UNKN	OK	1239.258	28.26	ND	35.97	86.28	
A11	SOD - RADIANT SYBR 2017		UNKN	OK	1316.749	28.17	ND	36.3	86.17	
A12	SOD - RADIANT SYBR 2017		UNKN	OK	1368.982	26.11	ND	36.09	86.28	
A13	SOD - RADIANT SYBR 2017	362	UNKN	OK	826.5135	26.90	ND	34.87	86.13	
A14	SOD - RADIANT SYBR 2017		UNKN	OK	1008.125	26.54	ND	33.27	86.03	
A15	SOD - RADIANT SYBR 2017		UNKN	OK	1071.519	26.49	ND	33.07	86.18	
A16	SOD - RADIANT SYBR 2017	363	UNKN	OK	342.972	28.29	ND	32.25	86.35	
B1	SOD - RADIANT SYBR 2017		UNKN	OK	375.696	28.19	ND	0	86.31	
B2	SOD - RADIANT SYBR 2017		UNKN	OK	214.756	29.03	ND	31.59	86.25	
B3	SOD - RADIANT SYBR 2018	364	UNKN	OK	2305.857	25.28	ND	32.59	86.25	
B4	SOD - RADIANT SYBR 2019		UNKN	OK	2549.829	25.13	ND	33.59	86.25	
B5	SOD - RADIANT SYBR 2020		UNKN	OK	2986.582	24.88	ND	34.59	86.25	
B3	SOD - RADIANT SYBR 2017	365	UNKN	OK	1801.332	25.67	ND	33.86	86.21	
B4	SOD - RADIANT SYBR 2017		UNKN	OK	1967.734	25.53	ND	32.93	86.31	
B5	SOD - RADIANT SYBR 2017		UNKN	OK	1883.642	25.52	ND	33.12	86.41	
B6	SOD - RADIANT SYBR 2017	366	UNKN	OK	931.869	26.71	ND	33.26	86.17	
B7	SOD - RADIANT SYBR 2017		UNKN	OK	727.116	27.10	ND	32.33	86.19	
B8	SOD - RADIANT SYBR 2017		UNKN	OK	828.458	26.90	ND	33.23	86.17	
B9	SOD - RADIANT SYBR 2017	367	UNKN	OK	348.439	28.26	ND	32.79	86.36	
B10	SOD - RADIANT SYBR 2017		UNKN	OK	312.327	28.44	ND	32.57	86.2	
B11	SOD - RADIANT SYBR 2017		UNKN	OK	324.748	28.37	ND	32.89	86.38	
B12	SOD - RADIANT SYBR 2018	368	UNKN	OK	324.748	28.38	ND	33.89	86.28	
B12	SOD - RADIANT SYBR 2018		UNKN	OK	324.748	28.38	ND	34.89	86.33	
B14	SOD - RADIANT SYBR 2020		UNKN	OK	324.748	28.38	ND	35.89	86.38	
B12	SOD - RADIANT SYBR 2017	369	UNKN	OK	2218.769	25.84	ND	33.21	86.26	
B13	SOD - RADIANT SYBR 2017		UNKN	OK	2209.117	25.35	ND	32.98	86.21	
B14	SOD - RADIANT SYBR 2017		UNKN	OK	2366.2943	25.28	ND	34.54	86.2	
B15	SOD - RADIANT SYBR 2017	370	UNKN	OK	1014.533	25.58	ND	32.27	86.14	
B16	SOD - RADIANT SYBR 2017		UNKN	OK	1095.239	25.46	ND	33.29	86.33	
C1	SOD - RADIANT SYBR 2017		UNKN	OK	1112.351	25.43	ND	32.63	86.41	
C2	SOD - RADIANT SYBR 2017	371	UNKN	OK	805.293	26.94	ND	30.66	86.28	
C3	SOD - RADIANT SYBR 2017		UNKN	OK	706.822	27.15	ND	30.85	86.42	
C4	SOD - RADIANT SYBR 2017		UNKN	OK	853.694	26.85	ND	30.41	86.23	
C5	SOD - RADIANT SYBR 2017	379	UNKN	OK	7893.555	25.09	ND	31.09	86.29	
C6	SOD - RADIANT SYBR 2017		UNKN	OK	7644.539	24.94	ND	30.95	86.3	
C7	SOD - RADIANT SYBR 2017		UNKN	OK	7690.931	24.91	ND	30.87	86.21	
A1	SOD - RADIANT SYBR 2017	380	UNKN	OK	7155.188	25.76	ND	28.49	86.65	
A2	SOD - RADIANT SYBR 2017		UNKN	OK	7275.83	25.62	ND	27.65	86.23	
A3	SOD - RADIANT SYBR 2017		UNKN	OK	7101.068	25.54	ND	0	86.45	
A4	SOD - RADIANT SYBR 2017	381	UNKN	OK	12860.656	24.81	ND	27.51	86.26	
A5	SOD - RADIANT SYBR 2017		UNKN	OK	11507.04	27.34	ND	28.56	86.15	
A6	SOD - RADIANT SYBR 2017		UNKN	OK	10902.198	28.03	ND	28.15	86.33	
A7	SOD - RADIANT SYBR 2017	382	UNKN	OK	454.313	28.33	ND	33.23	86.2	
A8	SOD - RADIANT SYBR 2017		UNKN	OK	434.4	28.34	ND	33.1	86.33	
A9	SOD - RADIANT SYBR 2017		UNKN	OK	605.783	28.23	ND	32.12	86.55	
A10	SOD - RADIANT SYBR 2017	383	UNKN	OK	458.976	28.31	ND	33.23	86.23	
A11	SOD - RADIANT SYBR 2017		UNKN	OK	270.279	28.68	ND	33.89	86.34	
A12	SOD - RADIANT SYBR 2017		UNKN	OK	338.145	28.81	ND	33.59	86.43	
A13	SOD - RADIANT SYBR 2017	384	UNKN	OK	1506.613	26.28	ND	31.52	86.51	
A14	SOD - RADIANT SYBR 2017		UNKN	OK	1446.257	26.16	ND	30.57	86.2	
A15	SOD - RADIANT SYBR 2017		UNKN	OK	1137.594	26.11	ND	30.59	86.19	
A16	SOD - RADIANT SYBR 2017	385	UNKN	OK	3162.386	26.73	ND	31.26	86.55	
B1	SOD - RADIANT SYBR 2017		UNKN	OK	2896.67	26.59	ND	0	86.25	
B2	SOD - RADIANT SYBR 2017		UNKN	OK	2755.836	26.58	ND	31.5	86.22	
B3	SOD - RADIANT SYBR 2017	386	UNKN	OK	3655.386	26.08	ND	33.71	86.34	
B4	SOD - RADIANT SYBR 2017		UNKN	OK	2981.45	26.12	ND	32.26	86.19	
B5	SOD - RADIANT SYBR 2017		UNKN	OK	3212.295	27.99	ND	31.88	86.47	

SAED000023(color)

Calculation

Gene of Interest		ATOX1												
			Unit											
1 Dalton = 1.66E-24 grams		1.66E-24	g											
Mass of base pair		650	Da											
Avg. Mass/base		309.25	Da											
Length of coding gene		651	bases											
Mass in Daltons		2.39E+04	Da	= number bases x avg. mass/base										
Mass in grams		4.81E-20	g	= mass in Da x mass of a Da in grams										
Mass in ug		4.81E-14	ug	= above / 10E-6										
Mass in ng		4.81E-11	ng/copy	= above x 10E3										
5/2/2018 18:00														
ID	Sample	Copy #	ul cDNA used	copies/ul cDNA	ug RNA used	ul cDNA made	ug RNA/ul cDNA	copies/ug RNA	Dilution Factor	copies/ul cDNA x DF	pg/ul RNA	Normalized/ng	Average	SD
360	EL1 Ont 72 hr	7883.556	3.000	2564.519	0.5	20	0.025	102580.75	10.000	1025807.467	4.40E+01	43.049	42.953	0.154
		7844.539	3.000	2648.180	0.5	20	0.025	101927.19	10.000	1019271.867	4.39E+01	42.775		
		7890.931	3.000	2563.644	0.5	20	0.025	102545.75	10.000	1025457.467	4.42E+01	43.035		
357	EL1 5 ug/ml Talc	6645.312	3.000	2215.104	0.5	20	0.025	88604.16	10.000	886041.600	3.82E+01	37.458	37.316	0.950
		6416.587	3.000	2139.862	0.5	20	0.025	85554.493	10.000	855544.933	3.69E+01	36.169		
		6746.584	3.000	2248.528	0.5	20	0.025	89941.12	10.000	899411.200	3.87E+01	38.033		
358	EL1 20 ug/ml Talc	2591.377	3.000	863.792	0.5	20	0.025	34551.693	10.000	345516.933	1.49E+01	18.677	18.422	0.236
		2582.923	3.000	860.974	0.5	20	0.025	34438.973	10.000	344389.733	1.47E+01	18.395		
		2605.857	3.000	841.886	0.5	20	0.025	33675.427	10.000	336754.267	1.45E+01	18.199		
359	EL1 100 ug/ml Talc	796.114	3.000	265.371	0.5	20	0.025	10614.853	10.000	106148.533	4.57E+00	4.577	4.393	0.344
		728.677	3.000	242.892	0.5	20	0.025	9715.6933	10.000	97156.933	4.18E+00	4.185		
		717.772	3.000	239.257	0.5	20	0.025	9570.2933	10.000	95702.933	4.12E+00	4.122		
393	Normal Ovarian Ont 72 hr	1554.313	3.000	518.104	0.5	20	0.025	20724.173	10.000	207241.733	8.93E+00	8.436	8.204	0.360
		1434.403	3.000	478.134	0.5	20	0.025	19125.373	10.000	191253.733	8.24E+00	7.786		
		1545.783	3.000	515.261	0.5	20	0.025	20610.44	10.000	206104.400	8.88E+00	8.390		
394	5 ug/ml	1223.238	3.000	407.746	0.5	20	0.025	16309.84	10.000	163098.400	7.03E+00	6.691	7.132	0.398
		1325.729	3.000	441.910	0.5	20	0.025	17676.387	10.000	176763.867	7.61E+00	7.252		
		1362.924	3.000	454.308	0.5	20	0.025	18172.32	10.000	181723.200	7.83E+00	7.455		
395	20 ug/ml	926.516	3.000	308.839	0.5	20	0.025	12353.541	10.000	123535.413	5.32E+00	4.794	5.086	0.255
		1008.254	3.000	336.085	0.5	20	0.025	13443.387	10.000	134433.867	5.79E+00	5.217		
		1015.192	3.000	338.397	0.5	20	0.025	13535.893	10.000	135358.933	5.83E+00	5.253		
396	100 ug/ml	392.144	3.000	130.715	0.5	20	0.025	5228.5867	10.000	52285.867	2.25E+00	2.252	2.277	0.094
		382.645	3.000	127.548	0.5	20	0.025	5101.9333	10.000	51019.333	2.20E+00	2.197		
		414.496	3.000	138.165	0.5	20	0.025	5526.6133	10.000	55266.133	2.38E+00	2.380		
379	FT33 Ont 72 hr	2691.837	3.000	897.279	0.5	20	0.025	34691.16	10.000	346911.600	1.49E+01	8.826	9.491	0.573
		2973.845	3.000	957.948	0.5	20	0.025	38317.933	10.000	383179.333	1.65E+01	9.750		
		2916.453	3.000	972.151	0.5	20	0.025	38888.04	10.000	388880.400	1.67E+01	9.895		
380	5 ug/ml Talc	1701.363	3.000	567.121	0.5	20	0.025	22684.84	10.000	226848.400	9.77E+00	4.938	5.244	0.377
		1867.672	3.000	622.557	0.5	20	0.025	24902.293	10.000	249022.933	1.07E+01	5.311		
		1962.982	3.000	654.327	0.5	20	0.025	26173.093	10.000	261730.933	1.13E+01	5.582		
381	20 ug/ml Talc	878.675	3.000	292.892	0.5	20	0.025	11715.667	10.000	117156.667	5.05E+00	5.046	4.589	0.462
		711.834	3.000	237.278	0.5	20	0.025	9491.12	10.000	94911.200	4.09E+00	4.088		
		811.926	3.000	270.642	0.5	20	0.025	10825.68	10.000	108256.800	4.66E+00	4.663		
382	100 ug/ml Talc	355.673	3.000	118.558	0.5	20	0.025	4742.3067	10.000	47423.067	2.04E+00	0.854	0.835	0.092
		332.300	3.000	110.783	0.5	20	0.025	4431.3333	10.000	44313.333	1.91E+00	0.798		
		355.334	3.000	118.445	0.5	20	0.025	4737.7867	10.000	47377.867	2.04E+00	0.854		
360	SKOV-3 Ont 72 hr	1454.403	3.000	484.771	0.5	20	0.025	19390.84	10.000	193908.400	8.35E+00	8.352	8.604	0.538
		1434.403	3.000	478.134	0.5	20	0.025	19125.373	10.000	191253.733	8.24E+00	8.237		
		1505.783	3.000	535.261	0.5	20	0.025	21410.44	10.000	214104.400	9.22E+00	9.222		
361	SKOV 5 ug/ml	1238.258	3.000	412.086	0.5	20	0.025	16523.44	10.000	165234.400	7.12E+00	3.518	3.714	0.185
		1316.740	3.000	438.916	0.5	20	0.025	17566.453	10.000	175664.533	7.56E+00	3.736		
		1368.982	3.000	456.327	0.5	20	0.025	18253.093	10.000	182530.933	7.86E+00	3.886		
362	20 ug/ml	826.514	3.000	275.505	0.5	20	0.025	11020.18	10.000	110201.800	4.75E+00	3.396	4.023	0.546
		1038.126	3.000	346.042	0.5	20	0.025	13841.667	10.000	138416.667	5.96E+00	4.266		
		1071.519	3.000	357.173	0.5	20	0.025	14286.92	10.000	142869.200	6.15E+00	4.403		
363	100 ug/ml	342.972	3.000	114.324	0.5	20	0.025	4572.96	10.000	45729.600	1.97E+00	0.847	0.768	0.210
		375.666	3.000	125.222	0.5	20	0.025	5008.88	10.000	50088.800	2.16E+00	0.928		
		214.756	3.000	71.585	0.5	20	0.025	2863.4133	10.000	28634.133	1.23E+00	0.530		
364	TOV112 Ont 72 hr	2305.857	3.000	768.619	0.5	20	0.025	30744.76	10.000	307447.600	1.32E+01	10.508	11.945	1.576
		2549.829	3.000	849.943	0.5	20	0.025	33997.72	10.000	339977.200	1.46E+01	11.651		
		2986.582	3.000	995.527	0.5	20	0.025	39821.093	10.000	398210.933	1.72E+01	13.647		
365	TOV112 5 ug/ml Talc	1801.332	3.000	600.444	0.5	20	0.025	24017.76	10.000	240177.600	1.03E+01	9.549	10.591	0.958
		1967.734	3.000	655.911	0.5	20	0.025	26236.453	10.000	262364.533	1.13E+01	10.866		
		1983.642	3.000	661.214	0.5	20	0.025	26448.56	10.000	264485.600	1.14E+01	10.959		
366	TOV112 20 ug/ml Talc	931.869	3.000	310.623	0.5	20	0.025	12424.92	10.000	124249.200	5.35E+00	5.139	4.572	0.965
		727.118	3.000	242.373	0.5	20	0.025	9694.9067	10.000	96949.067	4.16E+00	4.010		
		826.458	3.000	275.153	0.5	20	0.025	11046.107	10.000	110461.067	4.76E+00	4.569		
367	TOV112 100 ug/ml Talc	348.423	3.000	116.141	0.5	20	0.025	4645.64	10.000	46456.400	2.00E+00	0.740	0.697	0.039
		312.327	3.000	104.109	0.5	20	0.025	4164.36	10.000	41643.600	1.79E+00	0.663		
		324.748	3.000	108.249	0.5	20	0.025	4329.9733	10.000	43299.733	1.86E+00	0.689		
368	A2780 5 ug/ml	2218.788	3.000	739.589	0.5	20	0.025	29583.573	10.000	295835.733	1.27E+01	5.220	5.345	0.337
		2209.117	3.000	736.372	0.5	20	0.025	29454.893	10.000	294548.933	1.27E+01	5.197		
		2268.264	3.000	756.088	0.5	20	0.025	31843.924	10.000	318439.240	1.37E+01	5.619		
370	20 ug/ml	1014.503	3.000	338.178	0.5	20	0.025	13527.107	10.000	135271.067	5.83E+00	3.401	3.601	0.175
		1095.329	3.000	365.060	0.5	20	0.025	14603.187	10.000	146031.867	6.29E+00	3.672		
		1112.351	3.000	370.784	0.5	20	0.025	14831.347	10.000	148313.467	6.39E+00	3.729		
371	100 ug/ml	805.293	3.000	268.431	0.5	20	0.025	10737.24	10.000	107372.400	4.62E+00	2.315	2.357	0.215
		706.802	3.000	235.607	0.5	20	0.025	9424.2933	10.000	94242.933	4.06E+00	2.032		
		853.894	3.000	284.585	0.5	20	0.025	11382.587	10.000	113825.867	4.90E+00	2.456		
388	A2780 ont	3162.399	3.000	1054.129	0.5	20	0.025	42165.147	10.000	421651.467	1.82E+01	7.226	6.706	0.474
		2886.670	3.000	962.223	0.5	20	0.025	38488.933	10.000	384889.333	1.66E+01	6.596		
		2755.896	3.000	918.632	0.5	20	0.025	36745.28	10.000	367452.800	1.58E+01	6.297		



SAED000024(color)

1/7/2018 protein extraction Samples 356 ~ 386 ELISA

— Cells were seeded on 1-3-18 at a density of 1.2×10^6 cells per 150mm dish

— treat with talc (10mg/mL = 10^4 µg/mL) ← 1-4-18
100mg talc + 10mL DMSO → mix
↓
Johnson & Johnson, #300L7477, Lot# 13717RA)

$(x_1) \cdot (10^4 \mu g/mL) = (5 mL)(5 \mu g/mL) \rightarrow x_1 = 2.5 \mu L$
$(x_2) \cdot (10^4 \mu g/mL) = (5 mL)(20 \mu g/mL) \rightarrow x_2 = 10 \mu L$
$(x_3) \cdot (10^4 \mu g/mL) = (5 mL)(100 \mu g/mL) \rightarrow x_3 = 50 \mu L$

— after 72 hours treatment, collect cells and medium for ELISA
- Collect media and place in labeled 15mL tube for freezing
- Then add 10mL PBS
- Using cell scrape, scrape the bottom of the dish and rotate
- Remove the PBS and cell mixture and place into 15mL labeld tubes
- centrifuge 1800×g . 5min. 4°C.
- Suck out PBS. Cells will be collected at the bottom.
- place all tubes in -80°C freezer.

— Protein extraction
- 10X lysis buffer (BioVision #106-100-1 Lot# 21151061) diluted 1:10 with dd ultrapure H_2O
- 1 tablet protease inhibita added (Rocho Diagnostics #11836153001)
- Add 400µL (or 200~300µL) 1X lysis buffer to each tube (~ 1×10^7 cells)
  - incubated 30 min
  - Centrifuge 13000 rpm . 10min . 4°C
  - transfer supernatent to new 1.5mL tube = Protein (-80°C)

Sample ID	
356	EL1 Unt
357	EL1 5 ug/ml Talc
358	EL1 20 ug/ml Talc
359	EL1 100 ug/ml Talc
360	SKOV-3 unt
361	SKOV-3 5ug/ml
362	SKOV-3 20ug/ml
363	SKOV-3 100ug/ml
364	TOV112 Unt
365	TOV112 5 ug/ml Talc
366	TOV112 20 ug/ml Talc
367	TOV112 100 ug/ml Talc
368	A2780 Unt
369	A2780 5 ug/ml
370	A2780 20 ug/ml
371	A2780 100 ug/ml
379	FT33 unt
380	FT33 5ug/ml
381	FT33 20 ug/ml
382	FT33 100 ug/ml
383	NOE unt
384	NOE 5 ug/ml Talc
385	NOE 20 ug/ml Talc
386	NOE 100 ug/ml Talc

1/8/2018 BCA protein detection Assay
(Pierce cat # 23225)

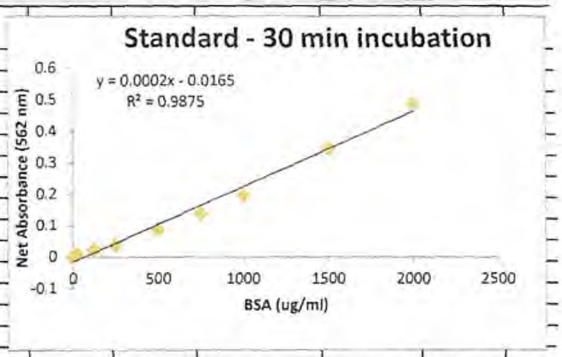
– Samples ID see pg 54

- (24 x 3 + 3 extra + 3 blank) = 78
  (24 x 3: samples; 78: wells)
- 200 µL per well = 200µL x 78 = 15600 µL
- 1 µL Reagent B per 50 µL Reagent A
  $\frac{15600}{50} = 312\ \mu L$
- 15600 µL Reagent A + 312 µL Reagent B

– Assay
- Add 10µL Sample to 3 wells
- Add 10µL of blank to 3 wells (whatever you lysed your cells with)
- Add 200µL of mix to each wells
- Mix, incubate at 37°C 30minuts
  * let plate to reach Room temp
- Read at 562 nm with spectrophotometer

– Stand Curve

Concentration (ug/ml)	OD1	OD2	OD3	Average	Corrected Avg
2000	0.5869	0.5848	0.5562	0.575967	0.4874
1500	0.4457	0.4211	0.3533	0.4334	0.344833
1000	0.3213	0.2774	0.2593	0.286	0.197433
750	0.2473	0.2199	0.2128	0.226667	0.1381
500	0.181	0.1759	0.1713	0.176067	0.0875
250	0.1328	0.1252	0.1256	0.127867	0.0393
125	0.1105	0.112	0.1116	0.111367	0.0228
25	0.1004	0.0941	0.0953	0.0966	0.008033
5	0.0845	0.0846	0.0941	0.087733	0
0	0.0881	0.0884	0.0892	0.088567	0



Compare results, with blank substracted. to the standard curve

SAED000027(color)

Compare results, with Blanks substracted,
To the standard curve which has been previously determined

30 minute incubation										
ID	OD1	OD2	OD3	- blank 1	- blank 2	-blank 3	ug/ml 1	ug/ml 2	ug/ml 3	Average (mg/ml)
TOV-112-C	0.2599	0.2418	0.223	0.1713	0.1532	0.1344	939.1667	848.6667	754.6667	0.8475
TOV112-5 ug	0.3313	0.3057	0.2243	0.2427	0.2171	0.1357	1296.167	1168.167	761.1667	1.232167
TOV112- 20ug	0.1986	0.1784	0.1741	0.11	0.0898	0.0855	632.6667	531.6667	510.1667	0.520917
TOV112-100 ug	0.4219	0.3751	0.3853	0.3333	0.2865	0.2967	1749.167	1515.167	1566.167	1.540667
SKOV-3-C	0.5228	0.5485	0.4355	0.4342	0.4599	0.3469	2253.667	2382.167	1817.167	2.317917
SKOV-3-5 ug	0.3486	0.2963	0.2995	0.26	0.2077	0.2109	1382.667	1121.167	1137.167	1.129167
SKOV-3-20 ug	0.5041	0.5503	0.4834	0.4155	0.4617	0.3948	2160.167	2391.167	2056.667	2.202667
SKOV-3-100 ug	0.5336	0.5384	0.511	0.445	0.4498	0.4224	2307.667	2331.667	2194.667	2.278
A2780-C	0.5125	0.5118	0.5274	0.4239	0.4232	0.4388	2202.167	2198.667	2276.667	2.200417
A2780-5 ug	0.5112	0.5135	0.5888	0.4226	0.4249	0.5002	2195.667	2207.167	2583.667	2.201417
A2780-20 ug	0.5432	0.5026	0.517	0.4546	0.414	0.4284	2355.667	2152.667	2224.667	2.188667
A2780-100 ug	0.5229	0.4448	0.377	0.4343	0.3562	0.2884	2254.167	1863.667	1524.667	1.880833
Normal ovarian-C	0.3136	0.2745	0.2506	0.225	0.1859	0.162	1207.667	1012.167	892.6667	1.0375
Normal Ovarian-Talc 5 ug	0.4511	0.4449	0.4128	0.3625	0.3563	0.3242	1895.167	1864.167	1703.667	1.821
Normal ovarian- Talc 20 ug	0.553	0.5402	0.5244	0.4644	0.4516	0.4358	2404.667	2340.667	2261.667	2.335667
Normal Ovarian-100 ug	0.4285	0.4308	0.4289	0.3399	0.3422	0.3403	1782.167	1793.667	1784.167	1.786667
Fallopian-C	0.3884	0.373	0.373	0.2998	0.2844	0.2844	1581.667	1504.667	1504.667	1.530333
Fallopian-5 ug	0.4075	0.4286	0.4376	0.3189	0.34	0.349	1677.167	1782.667	1827.667	1.7625
Fallopian-20ug	0.6752	0.67	0.6842	0.5866	0.5814	0.5956	3015.667	2989.667	3060.667	3.022
Fallopian-100 ug	0.2599	0.2418	0.223	0.1713	0.1532	0.1344	939.1667	848.6667	754.6667	0.8475
EL-1-C	0.5268	0.4749	0.4474	0.4382	0.3863	0.3588	2273.667	2014.167	1876.667	2.054833
EL-1-5 ug	0.269	0.2655	0.2811	0.1804	0.1769	0.1925	984.6667	967.1667	1045.167	0.999
EL-1-20 ug	0.5264	0.5212	0.5391	0.4378	0.4326	0.4505	2271.667	2245.667	2335.167	2.284167
EL-1-100 ug	0.5438	0.5555	0.5387	0.4552	0.4669	0.4501	2358.667	2417.167	2333.167	2.369667

40

1/11/2018 Catalase ELISA

Cayman chem. Cat # 707002

Catalytic Activity $2H_2O_2 \xrightarrow{CAT} O_2 + 2H_2O$

Peroxidatic Activity $H_2O_2 + AH_2 \xrightarrow{CAT} A + 2H_2O$

- Assay uses peroxidatic activity to determine enzymatic activity.
  - The enzyme with methanol in presence of optimal H_2O_2
  - The formaldehyde produced is measured colorimetrically with Purpald

Preparation

① - CAT assay buffer:
dilute 2ml of buffer concentrate with 18ml HPLC-grade water
- Store at 4°C, for 2 months

② - CAT sample buffer
dilute 5mL buffer with 45ml HPLC-grade water
- use to dilute the formaldehyde standards, control, samples
- Store at 4°C, 2 months

③ - CAT Formaldehyde Standard
The vial contains ~~4.5~~ 4.25 M formaldehyde
- Ready to use

④ - CAT (control)
Add 2ml of diluted Sample Buffer
Further dilute by taking 100ul + 1.9mL Sample buffer
- Only stable for 3days
- Reconstituted CAT (control) is stable for one month at -20°C

⑤ - CAT Potassium Hydroxide
Add 4ml of 10 mM KOH.
- Stable 3month at 4°C

SAED000029(color)

⑥ – CAT Hydrogen Peroxide
Dilute 40µl of CAT H_2O_2 with 9.96ml of HPLC-grade H_2O
* Stable for 2 hours

⑦ – CAT Purpald
Contains 4ml of purpald in 0.5M hydrochloric acid
Ready to use

⑧ – CAT Potassium Periodate
Contains 1.5ml of potassium Periodate in 0.5 M potassium hydro

– STANDARD
• Dilute 10µl of CAT Formaldehyde Standard with 9.99ml of diluted Sample Buffer to obtain a 4.25 mM formaldehyde stock solution
• Label tubes A – G, add accordingly ↓

Tube	Formaldehyde (µl)	Sample Buffer (µl)	Fi (µ
A	0	1,000	
B	10	990	
C	30	970	
D	60	940	
E	90	910	
F	120	880	
G	150	850	

* Final Formaldehyde concentrat in the 170µl reaction

Plate Set up

	1	2	3	4	5	6	7	8	9	10	11	12
A	A		368			364			379			
B	B		369			365			380			
C	C		370			366			381			
D	D		371			367			382			
E	E		360			383			356			
F	F		361			384			357			
G	G		362			385			358			
H	+	+	363			386			359			

A-G = standards
+ = Positive control

SAED000030(color)

- Performing the Assay
  - Formaldehyde Standard Wells - Add 100 µl of diluted Assay Buffer.
    - 30 µl of methanol ~~(tubes A-G)~~
    - 20 µl of standard (tubes A - G)
  - Positive Control Wells — Add 100 µl of diluted assay buffer
    - 30 µl of methand
    - 20 µl of diluted Catalase Control
  - Sample well — Add 100 µl of diluted Assay buffer
    - 30 µl of methand
    - 20 µl of sample to two well

- Stard Reaction by adding 20 µl of diluted Hydrogen Peroxide
  - ~~X~~ Note start time . Wave fast
  - Cover plate. incubate on shaker 20 minutes . Room temperture

- Add 30 µl of Potassium Hydroxide to each well to terminate Reaction
  - add 30 µl CAT purpald to each well
  - Cover plate . incubator for 10 minutes on shaker . Room temp.

- Add 10 µl CAT Potassium Periodate
  - Cover plate . 5 minutes . shaker . Room temp.

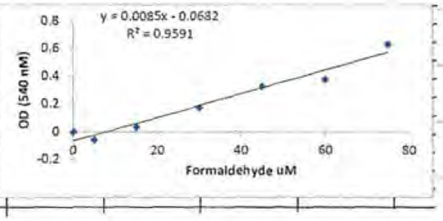
- Read the absorbance at 540

- Assay sensitive betwen 2~35 nmol/min/ml
- Catalase postive controls should give you absorbance ~0.2

— Calculation

- Calculate the average absorbances of each standard and samples
- Subtract the average of $OD_{standard\ A}$ from itself and all other standard. Sam
- Plot corrected absorbance of standards (y-axis) VS final formaldehyde concentration (uM) from equation obtained form standard curve

Standard	OD 1 (540 nm)	OD 2 (540 nm)	Average	Corrected Av	Formaldehyde (uM)
A	0.1312	0.1502	0.1407	0	0
B	0.1863	0.1786	0.18245	-0.0584167	5
C	0.2813	0.2705	0.2759	0.0350333	15
D	0.3882	0.4365	0.41235	0.1714833	30
E	0.5317	0.6039	0.5678	0.3269333	45
F	0.5171	0.7139	0.6155	0.3746333	60
G	0.903	0.8398	0.8714	0.6305333	75
Positive Control	0.858	0.7262	0.7921	0.5512333	



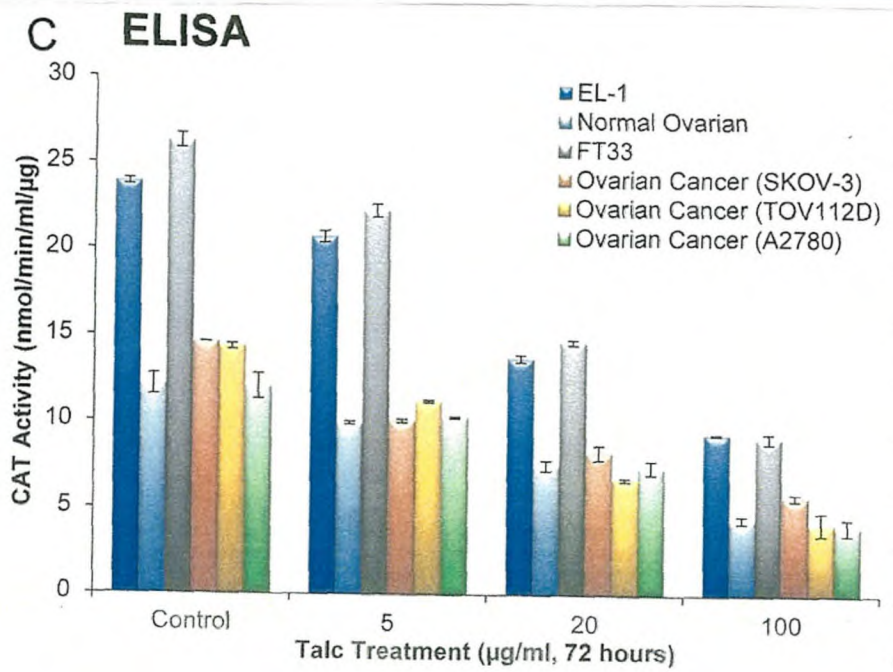
- Calculate the formaldehyde concentration of the samples using the equation obtaind from the linear regression of the standard curve substituting corrected absorbance values for each samples

$$\text{Formaldehyde (uM)} = \left[\frac{\text{Sample absorbance} - (\text{y-intercept})}{\text{slope}}\right] \times \frac{0.1\,\text{ml}}{0.02\,\text{ml}}$$

- Calculate the CAT activity of the sample using the following equation
  - One unit is defined as the amount of enzyme will cause the formation of 1.0 n mol of formaldehyde per minute at 25°C

$$\text{CAT activity} = \frac{\text{uM of Sample}}{20\,\text{min}} \times \text{Sample dilution} = \text{n mol/min/ml}$$

														2 ug protein used	
1/11/2018		OD 1	OD 2	OD 3	Corr 1	Corr2	Corr3	uM 1	uM 2	uM 3	nmol/min/ml	nmol/min/ml	nmol/min/ml	Average	SD
	BLANK	0.2598	0.2361	0.2267	0.240866667										
	A2780-C	0.3723	0.4053	0.3827	0.1314333	0.1644333	0.1418333	199.63333	232.63333	210.03333	9.98	11.63	10.50	11.07	0.80
	A2780-5 ug	0.3562	0.3855	0.3544	0.1153333	0.1446333	0.1135333	183.53333	212.83333	181.73333	9.18	10.64	9.09	9.13	0.06
	A2780-20 ug	0.2978	0.287	0.2857	0.0569333	0.0461333	0.0448333	125.13333	114.33333	113.03333	6.26	5.72	5.65	5.95	0.43
	A2780-100 ug	0.2153	0.211	0.225	-0.0255667	-0.0298667	-0.0158667	42.633333	38.333333	52.333333	2.13	1.92	2.62	2.27	0.49
	SKOV-3-C	0.4022	0.4504	0.4506	0.1613333	0.2095333	0.2097333	229.53333	277.73333	277.93333	11.48	13.89	13.90	13.89	0.01
	SKOV-3-5 ug	0.3839	0.3521	0.3489	0.1430333	0.1112333	0.1080333	211.23333	179.43333	176.23333	10.56	8.97	8.81	8.89	0.11
	SKOV-3-20 ug	0.3168	0.3037	0.3438	0.0759333	0.0628333	0.1029333	144.13333	131.03333	171.13333	7.21	6.55	8.56	6.88	0.46
	SKOV-3-100 ug	0.2118	0.2988	0.2177	-0.0290667	0.0579333	-0.0231667	39.133333	126.13333	45.033333	1.96	6.31	2.25	4.13	3.08
	TOV-112-C	0.4422	0.4475	0.4411	0.2013333	0.2066333	0.2002333	269.53333	274.83333	268.43333	13.48	13.74	13.42	13.61	0.19
	TOV112-5 ug	0.3742	0.3762	0.37371	0.1333333	0.1353333	0.1328433	201.53333	203.53333	201.04333	10.08	10.18	10.05	10.13	0.07
	TOV112- 20ug	0.2783	0.27531	0.26998	0.0374333	0.0344433	0.0291133	105.63333	102.64333	97.313333	5.28	5.13	4.87	5.21	0.11
	TOV112-100 ug	0.211	0.2314	0.2251	-0.0298667	-0.0094667	-0.0157667	38.333333	58.733333	52.433333	1.92	2.94	2.62	2.43	0.72
	Normal ovarian-C	0.3862	0.4055	0.3934	0.1453333	0.1646333	0.1525333	213.53333	232.83333	220.73333	10.68	11.64	11.04	11.16	0.68
	Normal Ovarian-Talc 5 ug	0.3492	0.3461	0.3444	0.1083333	0.1052333	0.1035333	176.53333	173.43333	171.73333	8.83	8.67	8.59	8.75	0.11
	Normal ovarian-Talc 20 ug	0.2987	0.28972	0.28702	0.0578333	0.0488533	0.0461533	126.03333	117.05333	114.35333	6.30	5.85	5.72	6.08	0.32
	Normal Ovarian-100 ug	0.2231	0.2298	0.23111	-0.0177667	-0.0110667	-0.0097567	50.433333	57.133333	58.443333	2.52	2.86	2.92	2.69	0.24
	Fallopian-C	0.7118	0.6988	0.6177	0.4709333	0.4579333	0.3768333	539.13333	526.13333	445.03333	26.96	26.31	22.25	26.63	0.46
	Fallopian-5 ug	0.6112	0.62301	0.6222	0.3703333	0.3821433	0.3813333	438.53333	450.34333	449.53333	21.93	22.52	22.48	22.22	0.42
	Fallopian-20ug	0.4534	0.4487	0.4222	0.2125333	0.2078333	0.1813333	280.73333	276.03333	249.53333	14.04	13.80	12.48	13.92	0.17
	Fallopian-100 ug	0.3245	0.3333	0.3198	0.0836333	0.0924333	0.0789333	151.83333	160.63333	147.13333	7.59	8.03	7.36	7.81	0.31
	EL-1-C	0.6554	0.6498	0.6511	0.4145333	0.4089333	0.4102333	482.73333	477.13333	478.43333	24.14	23.86	23.92	24.00	0.20
	EL-1-5 ug	0.5891	0.57891	0.5899	0.3482333	0.3380433	0.3490333	416.43333	406.24333	417.23333	20.82	20.31	20.86	20.57	0.36
	EL-1-20 ug	0.4332	0.4265	0.4544	0.1923333	0.1856333	0.2135333	260.53333	253.83333	281.73333	13.03	12.69	14.09	12.86	0.24
	EL-1-100 ug	0.3332	0.3345	0.3582	0.0923333	0.0936333	0.1173333	160.53333	161.83333	185.53333	8.03	8.09	9.28	8.06	0.05


C
ELISA
30
25
20
15
10
5
0
CAT Activity (nmol/min/ml/µg)
EL-1
Normal Ovarian
FT33
Ovarian Cancer (SKOV-3)
Ovarian Cancer (TOV112D)
Ovarian Cancer (A2780)
Control
5
20
100
Talc Treatment (µg/ml, 72 hours)

1/17/2018 CA-125 ELISA

RayBio. Cat # ELH-CA125

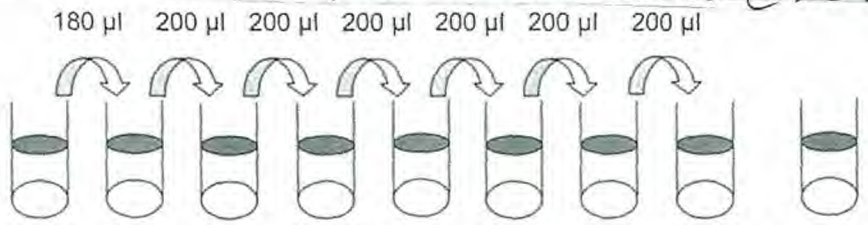
- This assay employs an antibody specific for human CA-125 coated on 96-well plate.
- CA125 present in sample is bound to the wells
- wash away unbound biotinylated antibody. HRP-conjugated streptavidin is pipetted to the wells,
- Wash again, color develop in proportion the amount of CA-125 bound
- Stop Solution, and measured at 450nm

- Preparation

- put all reagents and samples to room temperature (18-25°C).
- Assay ~~Att~~ Diluent diluted 5-fold with distilled H_2O.

- Sample dilution: 1X assay Diluent use for dilution of ~~serum~~ Samples.
  - The suggested dilution for normal serum/plasma is 2 fold
  - * levels of CA-125 may vary between different samples. Optimal dilution factors for each sample must be determined by the investigator

- Preparation of Standard: Briefly spin a vial of Item C.
  ~~add~~ Use the 400 U/ml standard solution to produce a dilution series (See below)



		Std1	Std2	Std3	Std4	Std5	Std6	Std7		Zero Standard
Diluent volume	Item C+ 400 µl	270 µl	400 µl	400 µl	400 µl	400 µl	400 µl	400 µl		400 µl
Conc.	1,000 U/ml	400 U/ml	133.3 U/ml	44.45 U/ml	14.81 U/ml	4.94 U/ml	1.65 U/ml	0.55 U/ml		0 U/ml

SAED000035(color)

- If the Wash Concentrate (20x) contains visible crystals, warm to Room temperature and mix gently.
  - Dilute 20ml of Wash buffer Concentrate into deionized or distilled water to yield 400ml of 1X Wash Buffer

- Briefly spin the Detection Antibody vial before use.
  - add 100µl of 1X assay diluent into the vial to prepare a detection
  - stored at 4°C for 5 days

- Briefly spin the HRP-Streptavidin concentrate vial and pipette up and down to mix gently.
  - diluted 800-fold with 1X Assay Diluent

- Assay Procedure

- Bring all reagents and samples to room temperature (18~25°C)

- Label removable 8-well strips as appropriate for you experiment

- Add 100µl of each standard and sample into appropriate
  - cover wells and incubate for 2.5 hours at room temp. gently sh

- Discard the solution and wash 4 times with 1X solution.
  - Wash with 300µl wash Buffer.
  - Complete removal of liquid
  - After the last wash, remove any remaining wash buffer by aspirating

- add 100µl of 1X prepared biotinylated antibody
  - Incubate for 1 hour at room temperature, gently shaking

- Discard the solution, Repeat the wash

SAED000036(color)

64

— 100μl of prepared Streptavidin Solution to each well
  • Incubate for 45 minutes, room temperature with gently shaking

— Discard the solution. Repeat the wash

— Add 100μl of TMB One-Step Substrate Reagent
  • Incubate 30 mins, room temperature in dark, gently shaking

— Add 50μl of stop solution to each well.

— Read at 450 nm immediately.

※ The minimum detectable dose of CA-125 was determined to be 0.6U/ml

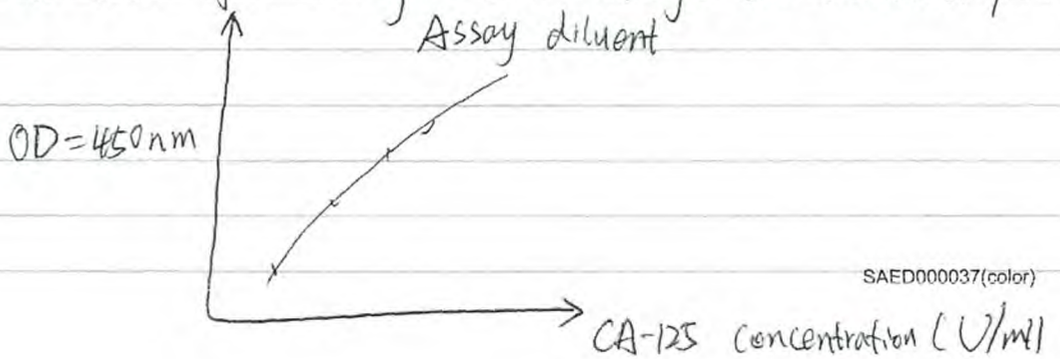
※ Intra-Assay CV%: <10%

※ Inter-Assay CV%: <12%

— Calculation of results
  • Calculate the mean absorbance for each set of duplicate standards, Controls and Samples and Subtract the average zero standard optical density.

  • plot the standard curve on log-log graph paper or using Sigma plot software, with standard concentration on the x-axis and absorbance on the y-axis

  • Draw the best-fit straight line through the standard points



– Recovery was determined by spiking various levels of CA-125 into the sample types listed below.

Sample Type	Average % Recovery	Range (%)
Serum	97.21	89-107
Plasma	76.88	68-85
Cell culture media	85.34	76-130

– Linearity

Sample Type		Serum	Plasma	Cell Culture Media
1:2	Average % of Expected	110.0	130.2	135.9
	Range (%)	99-118	119-138	125-142
1:4	Average % of Expected	107.5	126.4	92.99
	Range (%)	96-116	117-135	83-103

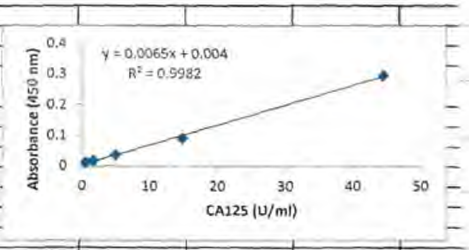
	1	2	3	4	5	6	7	8	9	10	11	12
A	A		TOV112			NOE						
B	B		TOV112 + Talc			NOE + Talc						
C	C		A2780			SKOV-3						
D	D		A2780 + Talc			SKOV-3 + Talc						
E	E		EL-1									
F	F		EL-1 + talc									
G	G		FTT									
H	Blank		FTT + talc									

Plate set up

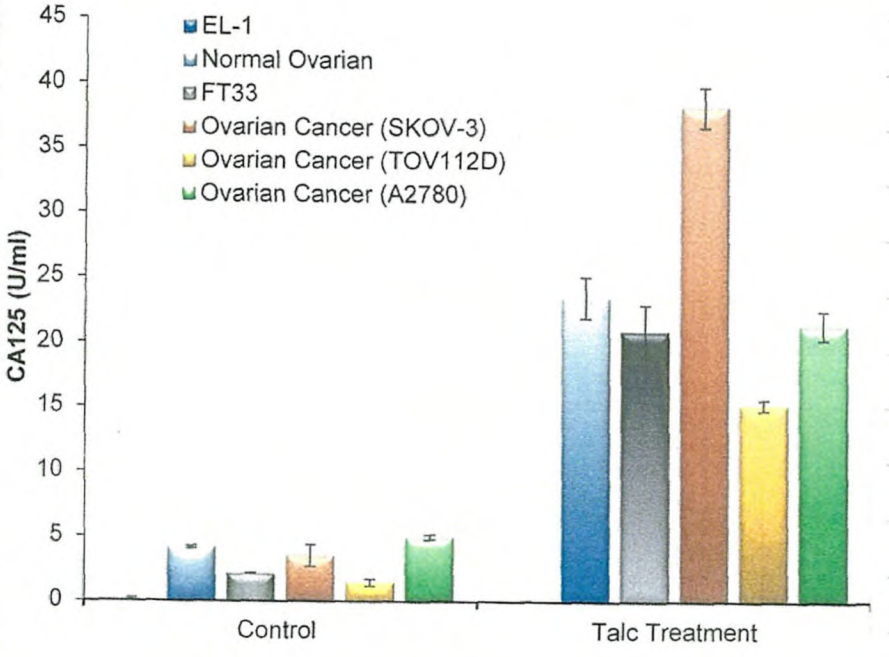
Talc Treatment : 100 µg/ml

Human CA-125 Standard curve.

1/17/2018					
Test media amounts for CA125 ELISA					
Standard (U/ml)	OD1 (450 nm)	OD2	Corrected OD1	Corrected OD2	Average
400	2.3856	2.3921	2.31895	2.32545	2.3222
133.3	1.1825	1.1458	1.11585	1.07915	1.0975
44.45	0.3643	0.358	0.29765	0.29135	0.2945
14.81	0.1593	0.1562	0.09265	0.08955	0.0911
4.94	0.1049	0.1009	0.03825	0.03425	0.03625
1.65	0.082	0.0861	0.01535	0.01945	0.0174
0.55	0.0769	0.0776	0.01025	0.01095	0.0106
Blank	0.0661	0.0672	0.06565		



in Media	0.0799	0.0885	0.0811	0.01325	0.02185	0.01445	0.01385							
mples	OD1	OD2	OD3	Corrected OD1	Corrected OD2	Corrected OD3	Corrected for Media OD1	Corrected for Media OD2	Corrected for Media OD3	CA125 U/ml	CA125 U/ml	CA125 U/ml	Average	
V112	0.096	0.0925	0.0939	0.02935	0.02585	0.02725	0.0155	0.012	0.0134	1.769230769	1.230769231	1.446153846	1.482051282	0.
V112+Talc	0.1849	0.1799	0.1843	0.11825	0.11325	0.11765	0.1044	0.0994	0.1038	15.44615385	14.67692308	15.35384615	15.15897436	
780	0.1179	0.1155	0.1172	0.05125	0.04885	0.05055	0.0374	0.035	0.0367	5.138461538	4.769230769	5.030769231	4.979487179	0.
780+ Talc	0.2216	0.2312	0.2172	0.15495	0.16455	0.15055	0.1411	0.1507	0.1367	21.09230769	22.56923077	20.41538462	21.35897436	1.
-1	0.0854	0.0862	0.0851	0.01875	0.01955	0.01845	0.0049	0.0057	0.0046	0.138461538	0.261538462	0.092307692	0.164102564	0.
-1+ Talc	0.0779	0.0795	0.0972	0.01125	0.01285	0.03055	-0.0026	-0.001	0.0167	-1.01538462	-0.76923077	1.953846154	0.056410256	1
lopian	0.0988	0.0985	0.0985	0.03215	0.03185	0.03185	0.0183	0.018	0.018	2.2	2.153846154	2.153846154	2.169230769	0.
lopian + Talc	0.2112	0.2355	0.2144	0.14455	0.16885	0.14775	0.1307	0.155	0.1339	19.49230769	23.23076923	19.98461538	20.9025641	2.
mal Ovarian	0.1111	0.1124	0.1114	0.04445	0.04575	0.04475	0.0306	0.0319	0.0309	4.092307692	4.292307692	4.138461538	4.174358974	0.
mal ovarian + Talc	0.222	0.234	0.255	0.15535	0.16735	0.18835	0.1415	0.1535	0.1745	21.15384615	23	26.23076923	23.46153846	2.
OV-3	0.1012	0.1103	0.1111	0.03455	0.04365	0.04445	0.0207	0.0298	0.0306	2.569230769	3.969230769	4.092307692	3.543589744	0.
OV3 +talc	0.3389	0.3211	0.3384	0.27225	0.25445	0.27175	0.2584	0.2406	0.2579	39.13846154	36.4	39.06153846	38.2	1.



SAED000040(color)

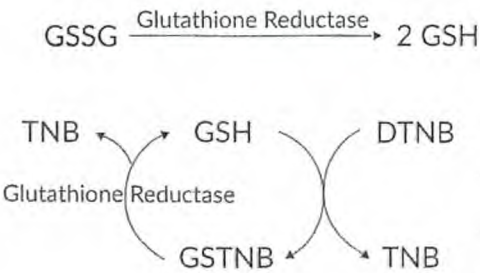
2/20/2018 Glutathione assay (Cayman chemical cat # 703002)

$Grx(SH)_2 + E\text{-}GS\text{-}SG\text{-}E \longrightarrow Grx \begin{cases} S\text{-}SG\text{-}E \\ SH \end{cases} \longrightarrow Grx\text{-}S_2 + E\text{-}GSH$

$Grx\text{-}S_2 + 2GSH \longrightarrow Grx\text{-}(SH)_2 + GSSG$

$GSSG + NADPH + H^+ \xrightarrow{(GR)} 2GSH + NADP^+$

- This kit measure the amount of total glutathione (GSH + GSSG)
- GSH is easily oxidized to the disulfide dimer GSSG. GSSG is produced during the reduction of hydroperoxides by glutathione peroxidase.
- This kit can also be used to measure only GSSG



GSH recycling

- Reagent Preparation
  - GSH MES Buffer (2X): 0.4 M 2-ethanesulphonic acid, 0.1 M phosphate. 2mM EDTA
    - Dilute 60mL of buffer with 60mL of HPLC-water
  - GSSG standard: 2mL of 25 µM GSSG in MES buffer
    - Ready to use
  - GSH Co-Factor Mixture: a lyophilized powder of NADP+ and glucose-6-phosphate
    - add 0.5mL HPLC-water
  - GSH Enzyme Mixture: glutathione reductase and glucose-6-phosphate in 0.2mL Buff
    - add 2mL of diluted MES Buffer

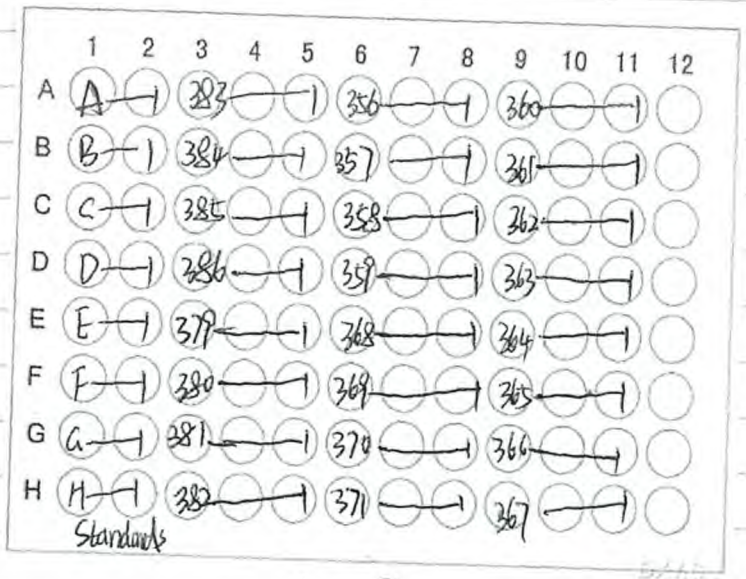
— GSH DTNB : a lyophilized powder of DTNB

Sample preparation :

- Collect cells by centrifugation . 1000~2000×g for 10min. 4°C
- The cell pellet can be homogenized in 1-2ml of cold buffer.
- Centrifuge 10000×g : 15min . 4°C
- Remove the supernatant , store on ice

Assay protocol :

— plate set up



Standard preparation

- Take eight clean test tubes and mark them A-H
- Aliquot the GSH Standard and MES buffer to each tube as described in table.

Tube	GSSG Standard (µl)	MES Buffer (µl)	Final Concentration (µM GSSG)	Equivalent Total GSH (µM)*
A	0	500	0	0
B	5	495	0.25	0.5
C	10	490	0.5	1.0
D	20	480	1.0	2.0
E	40	460	2.0	4.0
F	80	420	4.0	8.0
G	120	380	6.0	12.0
H	160	340	8.0	16.0

Performing the Assay

1. Add 50µL Standard (A-H)
2. Add 50µL samples to each of sample wells
3. Cover the plate with the plate cover
4. Prepare the assay and mix:

MES buffer	11.25mL
Cofactor Mixture	0.45mL
Enzyme Mixture	2.1mL
Water	2.3 mL
DTNB	0.45mL

5. Remove the plate cover and add 150µL of freshly prepared Assay Cocktail to each of wells.
   - Replace the plate cove
   - Incubate the plate in the dark on an orbital shaker
6. GSH concentration of samples determind by the End Point Method.
   - End point Method: Read Plate at 405-414nm after 25 minutes
   - Kinetic Method: Read the plate at 405-414nm at 5 minutes intervals for 30 minutes.

Analysis

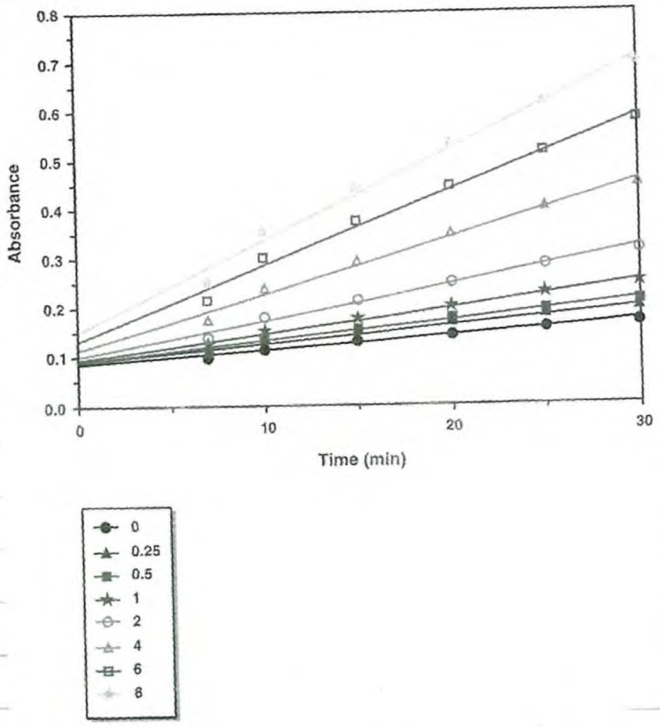
1. calculate the average absorbance from 25 minutes for each standard and sample
2. Subtract the absorbane value of the standard A from itself and all other values.
3. Plot the corrected absorbance values of each standard as a function of the concentration of GSSG or Total GSH
4. Calcute the values of GSSG or Total GSH for each sample from the standard curve

$$\text{Total GSH or GSSG} = \frac{A_{405} - \text{y-intercept}}{\text{slope}} \times 2 \times \text{Sample dilution}$$

If sample required deproteination multiply by "2" to account for the addition of MPA Reagent

Kinetic Method

1. Plot the average absorbance values of each standard and sample as a function of time and determine the slope for each curve



Plot of absorbance versus time to each standard

2. Plot the i-slopes of each standard as a function of concentration of GSSG

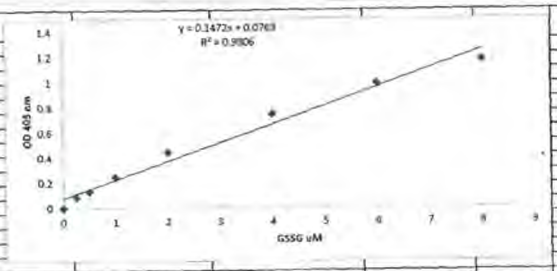
3. Calculate the values of GSSG for each sample from their respective slopes using the slope versus GSSG standard curve

$$\text{Total GSH or GSSG} = \frac{(\text{i-slope for sample}) - \text{y-intercept}}{\text{f-slope}} \times 2 \times \text{Sample dilution}$$

SAED000044(color)

≤ Inter-assay coefficient of variation is 3.5%
≤ Inter-assay coefficient is 1.6%

Standard GSSG uM	OD1	OD2	OD3	Average	Corrected
0	0.2398	0.2422	0.2396	0.240533333	0
0.25	0.3139	0.3119	0.3532	0.326333333	0.0858
0.5	0.3769	0.3713	0.3738	0.374	0.133466667
1	0.4877	0.4856	0.4845	0.485933333	0.2454
2	0.6801	0.6853	0.6807	0.682033333	0.4415
4	0.9867	0.99	0.9868	0.987833333	0.7473
6	1.2273	1.2338	1.2322	1.2311	0.990566667
8	1.4006	1.4119	1.4267	1.413066667	1.172533333



2/20/2018	using 30ug protein											
SAMPLE	OD1	OD2	OD3	uM GSSG	uM GSSG	uM GSSG	DF	x DF x2 for deprot	x DF x2 for deprot	x DF x2 for deprot	Average	SD
Normal ovarian-C	0.8465	0.9063	0.9107	0.605966667	0.665766667	0.670166667	2.6984127	3.270296296	3.593026455	3.616772487	3.6048995	0.016791
Normal Ovarian-Talc 5 ug	0.768	0.7602	0.7945	0.527466667	0.519666667	0.553966667	17.989418	2.846645503	2.804550265	2.989661376	2.8802857	0.0970324
Normal ovarian-Talc 20 ug	0.5931	0.589	0.5346	0.352566667	0.348466667	0.294066667	5.3968254	1.902740741	1.880613757	1.587026455	1.8901772	0.0156461
Normal Ovarian-100 ug	0.375	0.3655	0.3628	0.134466667	0.124966667	0.122266667	6.3968254	0.725693122	0.67442328	0.659851852	0.7000582	0.0362533
Fallopian-C	1.2553	1.2725	1.2698	1.014766667	1.031966667	1.029266667	7.3968254	5.476518519	5.569343915	5.554772487	5.5229312	0.0656375
Fallopian-5 ug	0.9852	0.9655	0.9746	0.744666667	0.724966667	0.734066667	8.3968254	4.018835979	3.912518519	3.96162963	3.9656772	0.0751778
Fallopian-20ug	0.7626	0.7666	0.7584	0.522066667	0.526066667	0.517866667	9.3968254	2.817502646	2.839089947	2.794835979	2.8282963	0.0152645
Fallopian-100 ug	0.4561	0.4121	0.4872	0.215566667	0.171566667	0.246666667	10.3968254	1.163375661	0.925915344	1.331216931	1.0408455	0.1679098
EL-1-C	1.1861	1.0981	1.2	0.945566667	0.857566667	0.959466667	12.3968254	5.103058201	4.628137566	5.178074074	4.8655979	0.3358196
EL-1-5 ug	0.9011	0.8911	0.911	0.660566667	0.650566667	0.670466667	13.3968254	3.564962963	3.510994709	3.618391534	3.5379788	0.0381613
EL-1-20 ug	0.711	0.871	0.8812	0.470466667	0.630466667	0.640666667	14.3968254	2.539026455	3.402518519	3.457566138	3.1330103	0.5105811
EL-1-100 ug	0.4555	0.544	0.511	0.214966667	0.303466667	0.270466667	15.3968254	1.160137566	1.637756614	1.459661376	1.3985471	0.3377277
A2780-C	1.366	1.411	1.366	1.125466667	1.170466667	1.125466667	17.3968254	6.07394709	6.316804233	6.07394709	6.1915794	0.1717259
A2780-5 ug	1.244	1.1482	1.122	1.003466667	0.907866667	0.881466667	18.3968254	5.415534392	4.898518519	4.757121693	5.0370415	0.3655854
A2780-20	0.9551	0.8792	0.799	0.714566667	0.638666667	0.558466667	19.3968254	3.856391534	3.446772487	3.01394709	3.4390369	0.2896444
A2780-100 ug	0.5111	0.5514	0.6331	0.270566667	0.310866667	0.392566667	20.3968254	1.460201058	1.677693122	2.118613757	1.7538026	0.1537901
SKOV-3-C	1.2297	1.211	1.295	0.989166667	1.070466667	1.054466667	21.3968254	5.338359788	5.777121693	5.690772487	5.6024176	0.3102515
SKOV-3-5 ug	1.0221	1.1087	1.098	0.781566667	0.868166667	0.857466667	22.3968254	4.217978836	4.685343915	4.627597884	4.5436401	0.330427
SKOV-3-20 ug	0.8972	0.9112	0.799	0.656666667	0.670666667	0.558466667	23.3968254	3.543915344	3.619470899	3.01394709	3.3857778	0.0534258
SKOV-3-100 ug	0.555	0.6113	0.599	0.314466667	0.370766667	0.358466667	24.3968254	1.697121693	2.000962963	1.934582011	1.8442233	0.2148482
TOV-112-C	1.1027	1.226	1.301	0.862166667	0.985466667	1.060466667	25.3968254	4.652962963	5.318391534	5.723153439	5.2981677	0.4705291
TOV112-5 ug	0.8932	0.9032	0.991	0.652666667	0.662666667	0.750466667	26.3968254	3.522328042	3.576296296	4.050137566	3.7962873	0.0381613
TOV112-20ug	0.6671	0.5982	0.555	0.426566667	0.357666667	0.314466667	27.3968254	2.30210582	1.93026455	1.697121693	1.9764974	0.2629315
TOV112-100 ug	0.444	0.3807	0.3775	0.203466667	0.139166667	0.136966667	28.3968254	1.098074074	0.805026455	0.739185185	0.8807619	0.207216

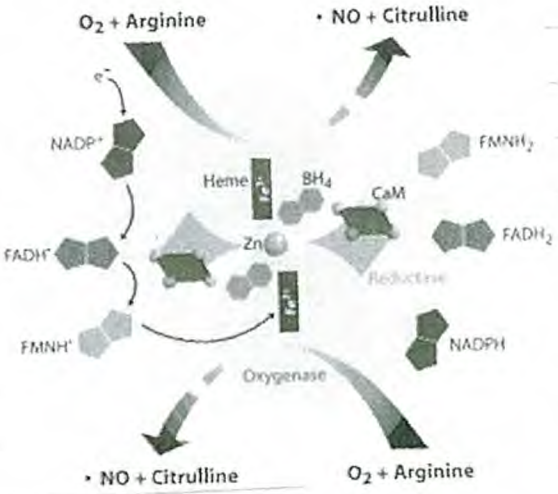
SAED000045(color)

Nitrate/Nitrite Assay Kit (LDH method)

2/25/2018

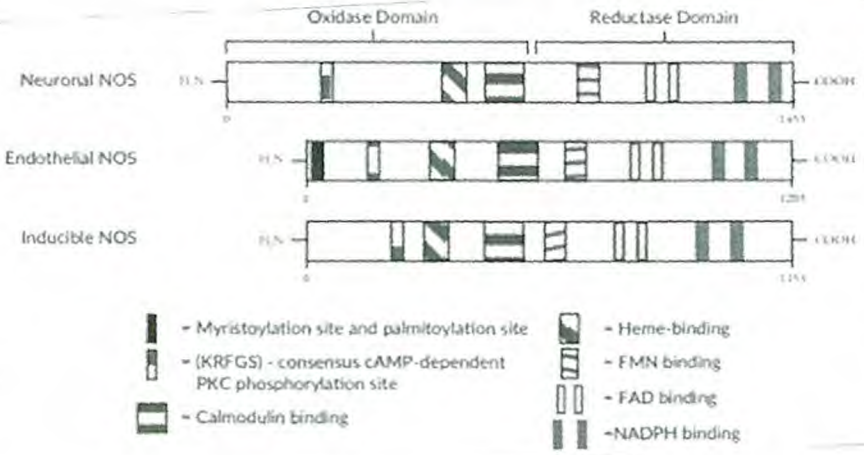
Cat # 760871

- Nitric Oxide (NO) is synthesized in biological systems by the Nitric Oxide Synthase (NOS)
- NOS is remarkably complex enzyme which acts on molecular oxygen, arginine, and NADPH to produce NO, citrulline, and NADP+
- This process requires five additional cofactors and two divalent cations.



Nitric Oxide Synthesis

Nitric Oxide Synthase Isoforms



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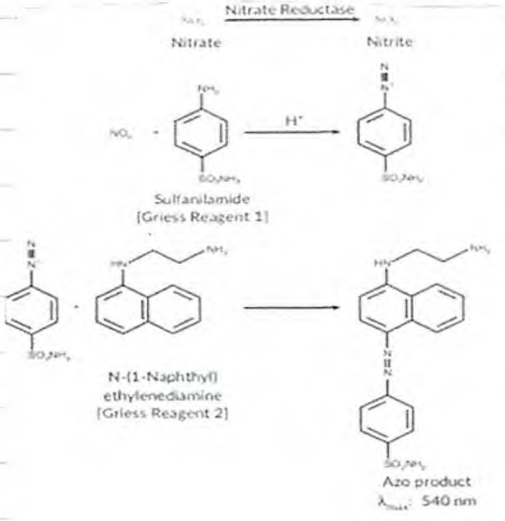


Chemistry of the Griess Reagents

- Preparation
  - Nitrate/Nitrite Assay Buffer
    - Dilute with 100ml UltralPure water
    - Store two months at 4°C
  - Nitrate Reductase (LDH method)
    - Reconstitute with 1.2mL of Assay Buffer
    - ~~X~~ keep on ice during ice
    - Store at -20°C, Freeze and thawing of this limited one time.
  - Lactate Dehydrogenase Cofactor Preparation
    - Reconstitute with 1.2ml of Assay Buffer
    - ~~X~~ keep on ice during ice
    - ~~X~~ Store at -20°C. Freeze and thawing of this one-time
  - Nitrate Standard
    - Reconstitute with 1.0ml of Assay Buffer
    - Store 4°C 4 months.

— Lactate Dehydrogenase
- Reconstitute the contents of the vial with 1.2mL of Assay buffer
- ※ keep on ice during use
- store at −20°C. one time use

— Griess Reagent R1 and R2
- Ready to use. no add
- store at 4°C

— NADPH
- Prepare a 1mM solution of NADPH in assay buffer.
- ≥1mM solution of NADPH will required.
- 0.017g = (833.4 g/mol)(0.001M)(x L) ⟹ x = 0.02046 = 20.4mL

※ Need media blance for each type medium
— RPMI have high Nitrate ~~ta~~ Levels.

—

Sample ID		
356	EL1 Unt	
357	EL1 5 ug/ml Talc	
358	EL1 20 ug/ml Talc	
359	EL1 100 ug/ml Talc	
360	SKOV-3 unt	
361	SKOV-3 5ug/ml	
362	SKOV-3 20ug/ml	
363	SKOV-3 100ug/ml	
364	TOV112 Unt	
365	TOV112 5 ug/ml Talc	
366	TOV112 20 ug/ml Talc	
367	TOV112 100 ug/ml Talc	
368	A2780 Unt	
369	A2780 5 ug/ml	
370	A2780 20 ug/ml	
371	A2780 100 ug/ml	
379	FT33 unt	
380	FT33 5ug/ml	
381	FT33 20 ug/ml	
382	FT33 100 ug/ml	
383	NOE unt	
384	NOE 5 ug/ml Talc	
385	NOE 20 ug/ml Talc	
386	NOE 100 ug/ml Talc	

- Standard preparation
  - In 1.5ml tube, add 0.9 mL Assay buffer and 0.1 mL reconstituted nitrate standard and vortex. (Now 200 μM)
  - Use this standard for the preparation of standard curve as described below

Well	Nitrate Standard (µl)	Assay Buffer (µl)	Final Nitrate Concentration (µM)*	Nitrate per well (nmoles)
A1, A2	0	60	0	0
B1, B2	5	55	5	1
C1, C2	10	50	10	2
D1, D2	15	45	15	3
E1, E2	20	40	20	4
F1, F2	25	35	25	5

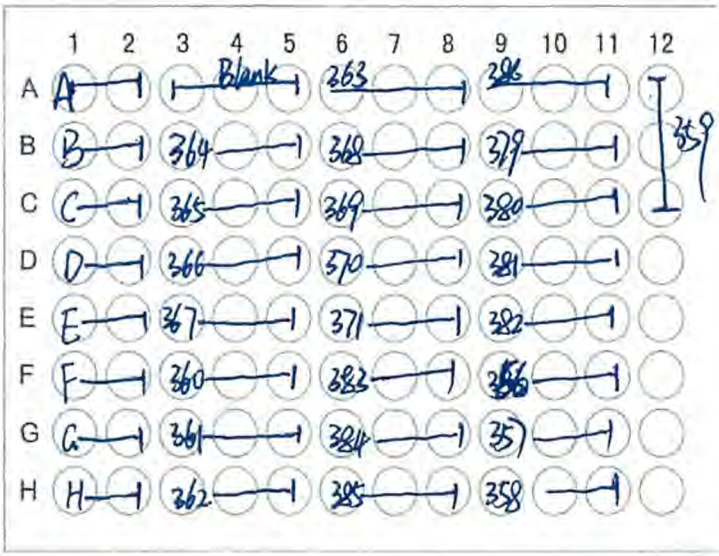
Assay

1. add standards to wells - as stated above
2. add 200μL of Assay Buffer to Blank well
3. add 60μL of sample the wells.
   - add 60μL media control
4. add 10μL of the freshly prepared NADPH solution (1mM) } standard samples
5. add 10μL of the Nitrate Reductase mixture.
6. Incubate at room temperature 40 minutes
7. add 10μL of the cofactors solution and 10μL of the LDH solution
8. Incubator at room temperature for 20 minutes
9. add 50 μL Griess Reagent R1
10. add 50 μL Griess Reagent R2
11. 10 minutes at room temperature
12. Read at 540nm or 550nm

- Calculation
  - Subtract the blanks
  - Plotting the standard Curve

$$\Delta\,[\text{Nitrate + Nitrite}]\,(\mu M) = \left[\frac{A_{540} - (\text{y-intercept})}{\text{Slope}}\right]\left[\frac{200\,\mu l}{V_{\text{sample}}\,(\mu l)}\right] \times \text{dilution}$$

Raw data & Plate set up



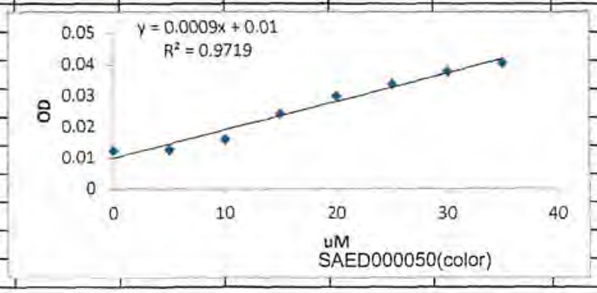
Plate

A- G : standards

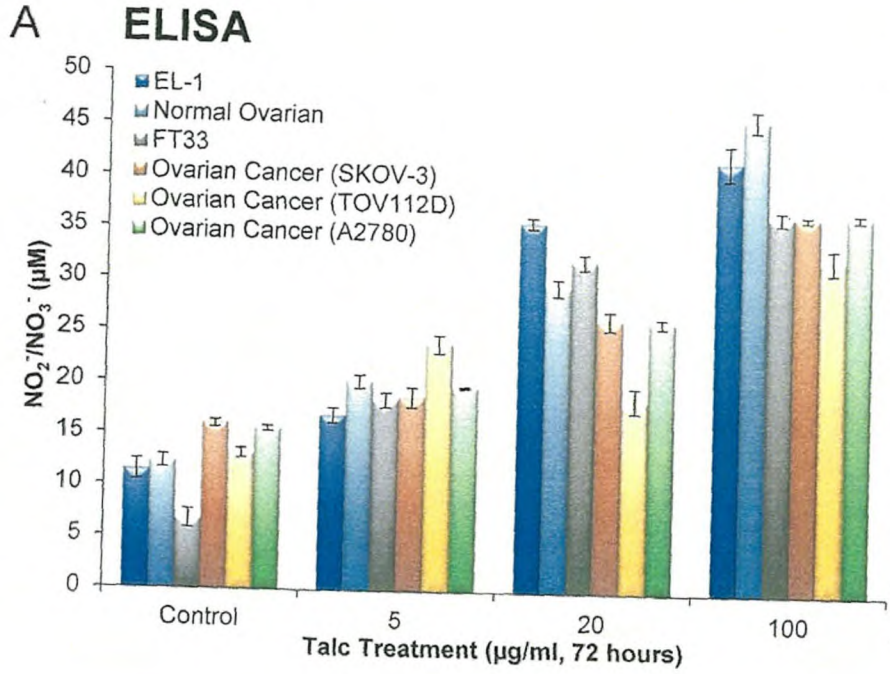
	STD	STD										
	1	2	3	4	5	6	7	8	9	10	11	12
	0.048	0.0481	0.0468	0.049	0.0493	[illegible]	[illegible]	[illegible]	0.08625	0.08553	0.08752	0.08112
	0.0484	0.0482	0.0578	0.0572	0.0592	0.05983	0.0589	0.0555	0.05113	0.05215	0.05228	0.08321
Raw	0.0491	0.0541	0.0662	0.0651	0.0681	0.06345	0.06427	0.06333	0.06141	0.06051	0.0631	0.08541
	0.0614	0.0582	0.06321	0.0617	0.0626	0.06945	0.06881	0.06883	0.07342	0.07435	0.0755	
data	0.0657	0.0652	0.0776	0.0734	0.0761	0.07883	0.0977	0.07843	0.07891	0.07833	0.0811	
	0.071	0.0677	[illegible]	[illegible]	[illegible]	0.0571	0.0565	0.0563	0.05531	0.05732	0.0566	
	0.0746	0.0722	[illegible]	[illegible]	[illegible]	0.06422	0.06334	0.06342	0.06132	0.06312	0.0604	
	0.0773	0.075	[illegible]	[illegible]	[illegible]	0.07112	0.07213	0.07321	0.07745	0.07569	0.07811	

Standard curve

CONC	STANDER W/O MEDIA			avg	corract
0	0.048	0.0481		0.04805	0.012417
5	0.0484	0.0482		0.0483	0.012667
10	0.0491	0.0541		0.0516	0.015967
15	0.0614	0.0582		0.0598	0.024167
20	0.0657	0.0652		0.06545	0.029817
25	0.071	0.0677		0.06935	0.033717
30	0.0746	0.0722		0.0734	0.037767
35	0.0773	0.075		0.07615	0.040517
lank	0.0346	0.0363	0.036	0.035633	0



samp		1	2	3	- blank	- blank	- blank	uM	uM	uM	avg	Sd
	HOSEpiC	0.0468	0.049	0.0493	0.011167	0.013367	0.013667	1.296296	3.740741	4.074074	3.907407	0.23570226
	TOV-112-C	0.0578	0.0572	0.0592	0.022167	0.021567	0.023567	13.51852	12.85185	15.07407	13.18519	0.47140452
	TOV112-5 ug	0.0662	0.0651	0.0681	0.030567	0.029467	0.032467	22.85185	21.62963	24.96296	23.90741	1.49278098
	TOV112- 20ug	0.06321	0.0617	0.0626	0.027577	0.026067	0.026967	19.52963	17.85185	18.85185	18.69074	1.18636804
	TOV112-100 ug	0.0776	0.0734	0.0761	0.041967	0.037767	0.040467	35.51852	30.85185	33.85185	32.35185	2.1213
	[illegible]	0.0597	0.0602	0.0603	0.024067	0.024567	0.024667	15.62963	16.18519	16.2963	16.03704	0.35717225
	[illegible]	0.0619	0.0657	0.06311	0.026267	0.030067	0.027477	18.07407	22.2963	19.41852	18.7463	0.95066578
	[illegible]	0.0699	0.06912	0.068777	0.034267	0.033487	0.033144	26.96296	26.0963	25.71519	26.33907	0.88231213
	[illegible]	0.0778	0.07812	0.07922	0.042167	0.042487	0.043587	35.74074	36.0963	37.31852	36.52963	1.11565737
	A2780-C	0.05983	0.0589	0.0555	0.024197	0.023267	0.019867	15.77407	14.74074	10.96296	13.36852	3.40196929
	A2780-5 ug	0.06345	0.06427	0.06333	0.027817	0.028637	0.027697	19.7963	20.70741	19.66296	19.72963	0.0942809
	A2780-20 ug	0.06945	0.06881	0.06888	0.033817	0.033177	0.033247	26.46296	25.75185	25.82963	26.1463	0.44783429
	A2780-100 ug	0.07883	0.0977	0.07843	0.043197	0.062067	0.042797	36.88519	57.85185	36.44074	36.66296	0.31426968
	Normal ovarian-C	0.0571	0.0565	0.0563	0.021467	0.020867	0.020667	12.74074	12.07407	11.85185	12.2963	0.62853936
	Normal Ovarian-Talc 5 ug	0.06422	0.06334	0.06342	0.028587	0.027707	0.027787	20.65185	19.67407	19.76296	20.20741	0.62853936
	Normal ovarian-Talc 20 ug	0.07112	0.07213	0.07321	0.035487	0.036497	0.037577	28.31852	29.44074	30.64074	29.47963	1.64205908
	Normal Ovarian-100 ug	0.08625	0.08553	0.08752	0.050617	0.049897	0.051887	45.12963	44.32963	46.54074	45.83519	0.99780624
	Fallopian-C	0.05113	0.05215	0.05228	0.015497	0.016517	0.016647	6.107407	7.240741	7.385185	6.746296	0.90352533
	Fallopian-5 ug	0.06141	0.06051	0.0631	0.025777	0.024877	0.027467	17.52963	16.52963	19.40741	18.46852	1.3277894
	Fallopian-20ug	0.07342	0.07435	0.0755	0.037787	0.038717	0.039867	30.87407	31.90741	33.18519	32.02963	1.63420234
	Fallopian-100 ug	0.07891	0.07833	0.0811	0.043277	0.042697	0.045467	36.97407	36.32963	39.40741	38.19074	1.7206265
	EL-1-C	0.05531	0.05732	0.0566	0.019677	0.021687	0.020967	10.75185	12.98519	12.18519	11.46852	1.01351972
	EL-1-5 ug	0.06132	0.06312	0.0604	0.025687	0.027487	0.024767	17.42963	19.42963	16.40741	16.91852	0.72282027
	EL-1-20 ug	0.07745	0.07569	0.07811	0.041817	0.040057	0.042477	35.35185	33.3963	36.08519	35.71852	0.51854497
	EL-1-100 ug	0.08112	0.08321	0.08541	0.045487	0.047577	0.049777	39.42963	41.75185	44.1963	41.81296	3.37054232



SAED000052(color)

4/8/2018 GSR Assay

Cayman Chem. Cat # 703202

- Glutathione reductase catalyzes NADPH dependent reaction of oxidized GSH (GSSG) to GSH.
- A high GSH/GSSG ratio crucial for protein against ox. stress

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2GSH + NADP^+$$

- Oxidation of NADPH to $NADP^+$ accompanied by a decrese in absorbance at 340 nm.

- Preparation

- GR Assay Buffer (10x)
  - dilute 2mL assay buffer with 18mL HPLC-grade water
  - store 2months. at 4°C
  - must be 25°C to be use in assay

- GR Sample Buffer (10x)
  - dilute 2mL Sample buffer with 18mL HPLC-grade water
  - use to dilute GR Control + GR samples
  - store 1 month. at 4°C

- GR Control
  - Aliquot and store at -20°C
  - transfer 10µL to tube plus 990µL sample Buffer
  - keep on ice, stable for 2hours
  - Will cause ~0.04 absorbance (V/min)

- GSSG
  - Ready to use

— GR – NAPDH

- Each vial enough for 40 vxns/wells
- Add 2ml HPLC-grade water + Vortex
- keep at Room Temp. store at 4°C, stable for 2 days
- No refreezing

— Assay Procedure

※ Final Volume of assay is 190μl/well, detect at 340nm

1. Add 120μl Assay Buffer and 20μl GSSG to 3wells → Blank
2. Add 100μl Assay Buffer and 20μl GSSG and 20μl dilute Control to 3 wells, → Control
3. For samples, add : 100μl Assay buffer
   20μl GSSG
   20μl samples

   *Amt GR added should cause absorbance ↓ between 0.08 ~ 0.1 /min

4. Initiate vxn : add 50μl NAPDH to All wells
   ※ as fast as possible
5. Shark plate for few seconds to mix.
6. Read absorbance at 340nm once every minute.
   - Get 5 time points
   - Initial reading Should be not above 1.2 or below 0.5

— Calculation

1. Δ absorbance per minute
   - plot absorbance values vs time
   - get slope

2. Determine rate of ΔA340/min for background/blance and subtract from rate of sample wells

SAED000054(color)

3. NADPH extinction coefficient = 0.00373 μM^{-1}
   - 1 unit = amt. enzyme that will cause oxidation at 1.0nmol
   - actual extinction is 0.00622 $\mu M^{-1} cm^{-1}$ – adjust from path of well.
   - NADPH to $NADP^+$ per min at 25°C

$$GR\ activity = \frac{\Delta A_{340/min}}{0.00373\ \mu M^{-1}} \times \frac{0.19\ mL}{0.02mL} \times dilution = nmol/min/ml$$

- Activity of 20~255 nmol/min/ml are in reproducible range
- this is equal to absorbance deverse of 0.008~0.1/min

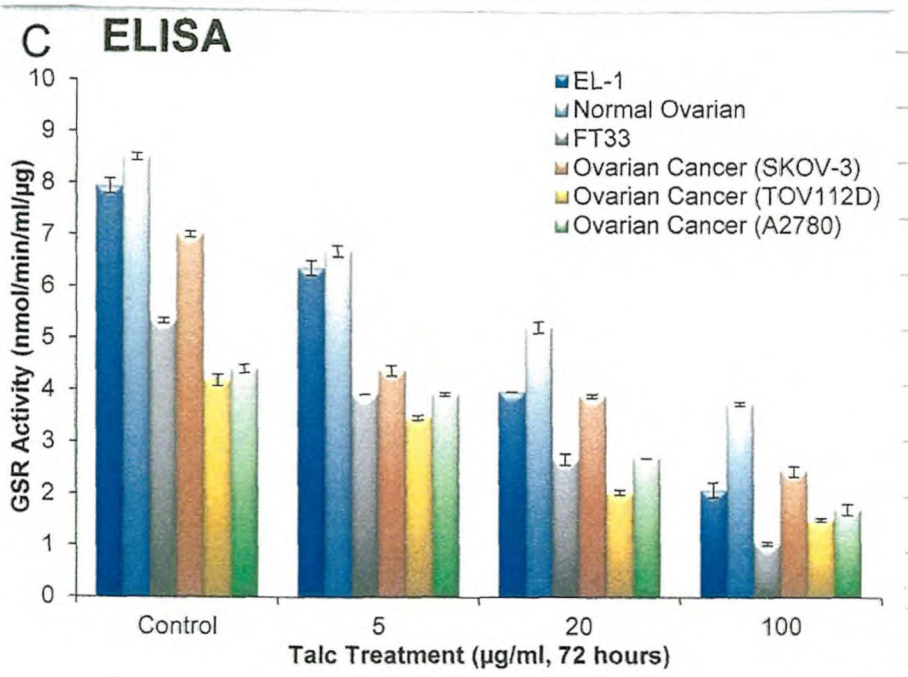
	1	2	3	4	5	6	7	8	9	10	11	12
A	Blank		382		366		370					
B	383		356		367		371					
C	384		351		360							
D	385		358		361							
E	386		359		362							
F	379		+	+	363							
G	380		364		368							
H	381		365		369							

Plate:

+ : positive control

4/8/2018																
sample diluted with buffer to 5g proteins																
ID		Abs 1	Abs 2	Slope		Abs 1	Abs 2	Slope		Abs 1	Abs 2	Slope		Average nmol/min /ml	SD	
Background				0				0				0		0.00000		
Positive Control		0.4857	0.3273	0.03168		0.4857	0.3257	0.032		0.4857	0.3257	0.032		0.03189		
	HOEpiC Unt	0.5185	0.5124	0.00122	3.107	0.5175	0.513	0.0009	2.292	0.512	0.5064	0.00112	2.853	2.57239	0.39621	
	TOV-112-C	0.5122	0.5038	0.00168	4.279	0.505	0.4969	0.00162	4.126	0.5012	0.4934	0.00156	3.973	4.20241	0.10806	
	TOV112-5 ug	0.4795	0.474	0.001375	3.502	0.4785	0.473	0.001375	3.502	0.4751	0.4697	0.00135	3.438	3.47017	0.04502	
	TOV112- 20ug	0.3936	0.3895	0.00082	2.088	0.3933	0.3893	0.0008	2.038	0.4818	0.4779	0.00078	1.987	2.03753	0.05094	
	TOV112-100 ug	0.3419	0.3389	0.0006	1.528	0.342	0.339	0.0006	1.528	0.437	0.4341	0.00058	1.477	1.51117	0.02941	
	SKOV-3-C	0.5791	0.5654	0.00274	6.979	0.5793	0.5656	0.00274	6.979	0.5699	0.556	0.00278	7.080	7.01251	0.05882	
	SKOV-3-5 ug	0.5757	0.5673	0.00168	4.279	0.5758	0.567	0.00176	4.483	0.5746	0.566	0.00172	4.381	4.38070	0.10188	
	SKOV-3-20 ug	0.5716	0.564	0.00152	3.871	0.5713	0.5638	0.0015	3.820	0.5694	0.5617	0.00154	3.922	3.89678	0.03602	
	SKOV-3-100 ug	0.5253	0.5203	0.001	2.547	0.5252	0.5204	0.00096	2.445	0.5214	0.5168	0.00092	2.343	2.44504	0.10188	
	A2780-C	0.5555	0.552	0.00175	4.457	0.511	0.5025	0.0017	4.330	0.511	0.5022	0.00176	4.483	4.42315	0.08187	
	A2780-5 ug	0.4888	0.481	0.00156	3.973	0.4881	0.4804	0.00154	3.922	0.4878	0.4801	0.00154	3.922	3.92225	0.03602	
	A2780-20 ug	0.4798	0.4745	0.00106	2.700	0.4768	0.4715	0.00106	2.700	0.4765	0.4712	0.00106	2.700	2.69973	0.00000	
	A2780-100 ug	0.4286	0.4254	0.00064	1.630	0.4287	0.4252	0.0007	1.783	0.428	0.4245	0.0007	1.783	1.70643	0.10806	

[redacted]																
	Background	Abs 1	Abs 2	Slope		Abs 1	Abs 2	Slope		Abs 1	Abs 2	Slope	Avg backgr	Average nmol/min /ml	SD	
383	Normal ovarian-C	0.2641	0.2598	0.0014333		0.2598	0.2586	0.0004		0.2593	0.2588	0.0001667	0.00028	0.00067		
384	Normal Ovarian-Talc 5 ug	0.0897	0.0731	0.00332	8.456	0.0792	0.0624	0.00336	8.558	0.0771	0.0597	0.00348	8.863	8.50670	0.072037688	
385	Normal ovarian- Talc 20 ug	0.5085	0.495	0.0027	6.877	0.501	0.4877	0.00266	6.775	0.5011	0.4881	0.0026	6.622	6.69839	0.10806	
386	Normal Ovarian-100 ug	0.4879	0.4777	0.00204	5.196	0.4873	0.4769	0.00208	5.298	0.4831	0.473	0.00202	5.145	5.22118	0.10806	
379	Fallopian-C	0.4622	0.455	0.00144	3.668	0.4447	0.4374	0.00146	3.718	0.4467	0.4393	0.00148	3.769	3.74397	0.03602	
380	Fallopian-5 ug	0.4983	0.4879	0.00208	5.298	0.4751	0.4646	0.0021	5.349	0.4692	0.4586	0.00212	5.399	5.34853	0.05094	
381	Fallopian-20ug	0.4286	0.4209	0.00154	3.922	0.4333	0.4258	0.0015	3.820	0.4318	0.4241	0.00154	3.922	3.92225	0.00000	
382	Fallopian-100 ug	0.4299	0.4245	0.00108	2.751	0.4316	0.4264	0.00104	2.649	0.4318	0.4267	0.00102	2.598	2.67426	0.10806	
356	EL-1-C	0.4245	0.4225	0.0004	1.019	0.4311	0.4293	0.00036	0.917	0.4318	0.4297	0.00042	1.070	1.04424	0.03602	
357	EL-1-5 ug	0.4363	0.4205	0.00316	8.048	0.4377	0.4222	0.0031	7.895	0.4377	0.4223	0.00308	7.845	7.94638	0.14408	
358	EL-1-20 ug	0.4347	0.422	0.00254	6.469	0.4349	0.4225	0.00248	6.316	0.4349	0.4226	0.00246	6.265	6.36729	0.14408	
359	EL-1-100 ug	0.4298	0.422	0.00156	3.973	0.4301	0.4224	0.00154	3.922	0.4318	0.424	0.00156	3.973	3.97319	0.00000	
		0.4264	0.4225	0.00078	1.987	0.4311	0.4269	0.00084	2.139	0.4278	0.4235	0.00086	2.190	2.08847	0.14408	



SAED000057(color)

5/14/2018 Glutathione Peroxidase Assay Kit
(Cayman chemical Cat # 703102)

— GPx Catalyzes the reduction of hydroperoxidases, including H_2O_2 by reduced GSH, protecting cell form oxidative damage.
- All are tetramelo of 4 identical subunit.
- Each subunit Contains a selenocysteine in active site which paitapates directly in the $2e^-$ veduction of peroxide substrate
  — GSH used as e^- donor to regenerate veduced form of selenocysteine

$$R\text{-}O\text{-}O\text{-}H + 2GSH \xrightarrow{GPx} R\text{-}OH + GSSG + H_2O$$

$$GSSG + NADPH + H^+ \xrightarrow{GR} 2\,GSH + NADP^+$$

- Oxidation of NADPH to $NADP^+$ accompanied by a decrease in absorbance at 340nm

Reagent Preparation

1. GPx assay buffer (10x), 3mL/vial
   - Add 27mL HPLC-H_2O to contents of vial
   - Store 4°C, 6months

2. GPx Sample Buffer (10x)
   - dilute 2mL Concentrate ~ 18mL HPLC-H_2O
   - Use to dilute contrd and samples
   - Store 4°C, Stable for 1 month

3. Glutathine Peroxidase (Control) — 50µl bovine erythrogle GPx
   - Aliquot and store at -20°C
   - Transfer 10µl to tube plus 490µl Sample buffer — on ice
   - Stable 4 hours . No freezing.
   - Absorbance by 0.051 A/min

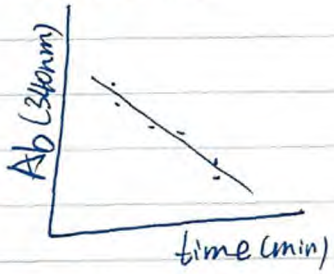
4. GPx . Co. Substrate mixture — Vial good for 40wells
   - Vial has NADPH, GSH, GR
   - Add 2ml 25°C while in use
   - store 4°C , 2days, No refreezing

5. GPx Cumene Hydroperoxide — ready to use
   - -20°C storage

※ Final Volume is 190µl/well
   - Detect at 340nm

— Assay
1. add 120µl assay buffer , 50µl co-substrate mixture to 3wells
   - Blank / background
2. Positive Contrd
   - 100µl assay buffer.
   - 50µl co-substrate mix.
   - 20µl diluted GPx Control to 3wells
3. Samples.
   - add 100µl assay buffer
   - 50µl co-substrate mix
   - 30µl Sample
4. Initiate rxn by adding 20µl Cumene hyoroperoxide per well as fast as possible
   - Shark to mix

5. Detect at 340nm Once every minute, at least 5 time point
   - Inital absorbance not above 1.2 or belowe 0.5



- Calculatione
  1. Determine ΔA 340nm per minuts
     . get slope
  2. Determine rate of background, subtract from rate of samples.
  3. Activity

$$GPx\ activity\ (nmol/min/mL) = \frac{\Delta A_{340}/min}{0.00373\,\mu M^{-1}} \times \frac{0.19mL}{0.02mL}$$

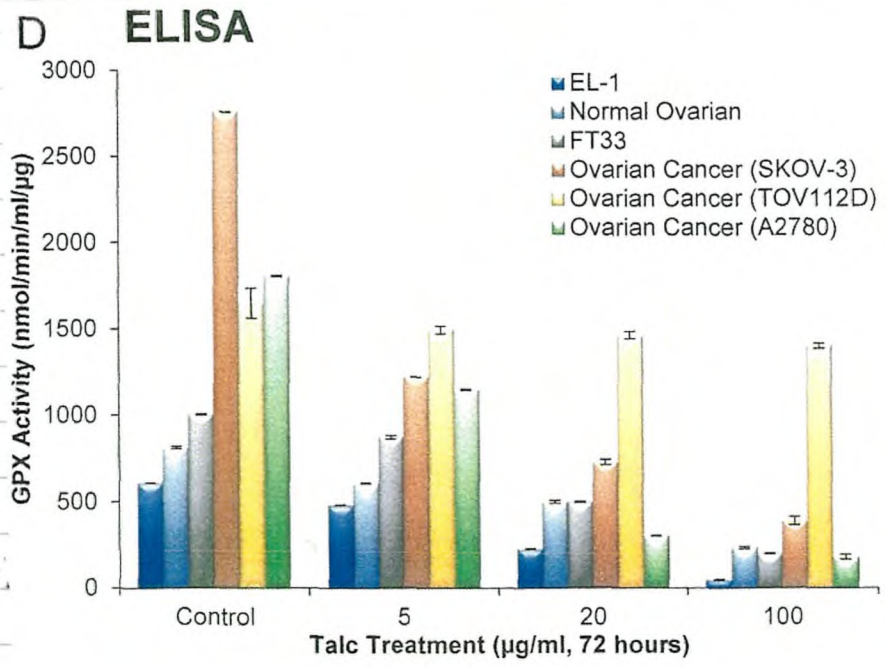
(0.19mL: Final Volume; 0.02mL: Sample Volume)

Sample with GPx activity in range of 50.344 ~~nmol~~ nmol/min/mL are in reproducible range
= to activity of 0.02 to 0.135/min ↓ in absorbance

5/14/2018	Titrate GPX							
	Slope	Slope	Abs Value	Abs Value	nmol/min/ml	nmol/min/ml	Average	Corrected
Background	-6.665	-5.71	6.665	5.71	16975.2011	14542.8954	15759.0483	
Pos Control	-40.481667	-41.146667	40.481667	41.146667	103103.441	104797.141	103950.291	88191.243
5ug - 394	-10.506667	-10.505	10.506667	10.505	26759.6076	26755.3619	26757.4848	10998.4365
10ug - 394	-16.908333	-16.928333	16.908333	16.928333	43064.1189	43115.0572	43089.5881	27330.5398

4/14/2018																
	Slope	Slope	Slope	Abs Value	Abs Value	Abs Value	Corrected	Corrected	Corrected	nmol/min/ml	nmol/min/ml	nmol/min/ml	Average	SD	per ug	SD
Background	-6.116667	-6.276667		6.116667	6.276667		6.196667			15578.6425	15986.1492					
Positive Control	-37.756667	-37.748333		37.756667	37.748333											
Normal ovarian-C	-6.834	-6.787	-6.795	6.834	6.787	6.795	0.637333	0.590333	0.598333	1623.23418	1503.529088	1523.904424	811.9162577	64.0453807	162.38325	6.40454
Normal Ovarian-Talc 5 ug	-6.673333	-6.696667	-6.68	6.673333	6.696667	6.68	0.476666	0.5	0.483333	1214.02869	1273.458445	1231.008981	607.2560097	30.61096	121.45120	3.06110
Normal ovarian- Talc 20 ug	-6.5898	-6.663333	-6.638333	6.5898	6.663333	6.638333	0.393133	0.466666	0.441666	1001.27708	1188.559517	1124.886595	500.8593719	95.22628	100.17187	9.52263
Normal Ovarian-100 ug	-6.381667	-6.346667	-6.341667	6.381667	6.346667	6.341667	0.185	0.15	0.145	471.179625	382.0375335	369.3029491	235.6623123	55.50877	47.13246	5.55098
Fallopian-C	-6.985	-6.961667	-6.9576	6.985	6.961667	6.9576	0.788333	0.765	0.760933	2007.81863	1948.391421	1938.03311	1004.299783	37.65835	200.85796	3.76583
Fallopian-5 ug	-6.88333	-6.8756	-6.818333	6.88333	6.8756	6.818333	0.686663	0.678933	0.621666	1748.87359	1729.185925	1583.331635	874.7476292	90.42973	174.94953	9.04297
Fallopian-20ug	-6.59	-6.576	-6.5923	6.59	6.576	6.5923	0.393333	0.379333	0.395633	1001.78646	966.1296247	1007.64437	501.0910471	22.46924	100.21821	2.24692
Fallopian-100 ug	-6.353333	-6.376667	-6.3665	6.353333	6.376667	6.3665	0.156666	0.18	0.169833	399.015282	458.4450402	432.5505362	199.5925573	29.79663	39.91851	2.97966
EL-1-C	-6.675	-6.678333	-6.6767	6.675	6.678333	6.6767	0.478333	0.481666	0.480033	1218.2744	1226.763271	1222.604155	609.3772149	4.24472	121.87544	0.42447
EL-1-5 ug	-6.575	-6.58933	-6.5946237	6.575	6.58933	6.5946237	0.378333	0.392663	0.3979567	963.582708	1000.080027	1013.562641	481.9903322	25.85804	96.39807	2.58580
EL-1-20 ug	-6.37536	-6.378333	-6.396667	6.37536	6.378333	6.396667	0.178693	0.181666	0.2	455.11622	462.6882038	509.383378	227.6581099	29.39019	45.53162	2.93902
EL-1-100 ug	-6.2334	-6.228301	-6.231667	6.2334	6.228301	6.231667	0.036733	0.031634	0.035	93.5558981	80.5691689	89.14209115	46.79544906	21.17796	9.35909	2.11780

4/23/2018																
	Slope	Slope	Slope	Abs Value	Abs Value	Abs Value	Corrected	Corrected	Corrected	nmol/min/ml	nmol/min/ml	nmol/min/ml	Average	SD	per ug	SD
Background	-4.89	-4.623333	-4.568333	4.89	4.623333	4.568333	4.595833									
Control	-23.293333	-23.921667	-24.401667	23.293333	23.921667	24.401667	18.6975	19.325834	19.805834	47620.9786	49221.29303	50443.81				
A2780-C	-5.318333	-5.293333	-5.39	5.318333	5.293333	5.39	0.7225	0.6975	0.794167	1840.14745	1776.474531	2022.68	1808.31	45.02	180.83	4.50235551
A2780-5 ug	-5.303333	-5.058333	-5.035	5.303333	5.058333	5.035	0.7075	0.4625	0.439167	1801.9437	1177.949062	1118.52	1148.24	42.02	114.62	4.202138445
A2780-20 ug	-4.718333	-4.575	-4.711667	4.718333	4.575	4.711667	0.1225	-0.020833	0.115834	311.997319	-53.05991957	295.02	303.51	12.01	30.35	1.200508073
A2780-100 ug	-4.66	-4.723333	-4.616667	4.66	4.723333	4.616667	0.064167	0.1275	0.020834	163.428016	324.7319035	53.06	180.41	136.63	18.04	13.66283167
SKOV-3-C	-5.675	-5.683333	-5.648333	5.675	5.683333	5.648333	1.079167	1.0875	1.0525	2748.54866	2769.772118	2680.63	2759.16	15.01	275.92	1.500725139
SKOV-3-5 ug	-5.073333	-5.08	-5.056667	5.073333	5.08	5.056667	0.4775	0.484167	0.460834	1216.15282	1233.13311	1173.71	1224.64	12.01	122.46	1.200688167
SKOV-3-20 ug	-4.843333	-4.846667	-4.956667	4.843333	4.846667	4.956667	0.2475	0.250834	0.360834	630.36193	638.8533512	919.01	729.41	164.26	72.94	16.42570894
SKOV-3-100 ug	-4.743333	-4.846667	-4.656667	4.743333	4.846667	4.656667	0.1475	0.250834	0.060834	375.670241	638.8533512	154.94	389.82	242.27	38.98	24.22672523
TOV-112-C	-6.65	-6.17	-6.628333	6.65	6.17	6.628333	2.054167	1.574167	2.0325	5231.79263	4009.27252	5176.61	4805.89	864.45	480.59	86.4452258
TOV112-5 ug	-5.846667	-5.956667	-5.833333	5.846667	5.956667	5.833333	1.250834	1.360834	1.2375	3185.77024	3465.931099	3151.81	3267.84	222.12	326.78	22.21174058
TOV112- 20ug	-5.746667	-5.965	-5.655	5.746667	5.865	5.655	1.150834	1.269167	1.059167	2931.07855	3232.462869	2697.61	2953.72	213.11	295.37	21.31108938
TOV112-100 ug	-5.578333	-5.463333	-5.493333	5.578333	5.463333	5.493333	0.9825	0.8675	0.8975	2502.34584	2209.450402	2285.86	2332.55	153.08	233.26	15.30800873



SAED000062(color)

5/18/2018

MPO ELISA

Northwast Life Science
Cat # NWK-mpo03

Myeloperoxiase – MPO

-Test principle

$$H_2O \xrightarrow[Cl^-]{MPO} HOCl$$

$$HOCl + TauNH_2 \longrightarrow TauNHCl + H_2O$$

Test principle:

$$TauNHCl + 2TNB \longrightarrow DTNB + Cl^- + TauNH_2$$

(Baseline Abs412 is Decreased)

- HOCl is rapidly trapped by β-amino acid taurine to form the stable oxidant taurine chloramine.
- Prevents accumulation of HOCl that can deactivate MPO
- After incubation for specific time, the MPO catalyzed reaction is stopped by add catlase to eliminate hydrogen peroxide.
- Taurine choramine is then allowed to react with TNB, a yellow complex with maximal absorbance at 412nm.
- 1 unit of MPO activity defined the amount of enzyme that can catalyze sufficient HOCl production resulting in formation of 1nmol TauNHCl at pH 6.5, 25°C over 30 minutes in presence 100mM Chloride and 100μM H_2O_2.

Reagents: Warm Kit ~ 2 hours, room temperature

- Assay Buffer: ready to use

- H_2O_2 reagent: Mix 12μl of solution from the Hydrogen Peroxide Vial into 4988μl Assay Buffer
  - mix + incubate 60min at roomtemp. before use
  - must be used within 3hours.
  - Label as working H_2O_2 solution

- Catalase reagent : Reconstitute the catalase Reagent with 20 mL of Assay Buffer
  - Mix and labeled.

- TNB Reagent : Add 28mL Assay Buffer to the TNB vial.
  - Mix and labeled
  - stand at room temp for at least 5 minutes before using

※ Working H_2O_2, Catalase and TNB Solution are stable for 3hours after dilution and must be used ~~with~~ within that time

- Cells preparation :
  - Cells from pg 33
  - using medium.

- Assay Protocols:

  - "Zero MPO Standard" is created by substituting Assay Buffer for sample.
  - the baseline for TNB absorbance Abase used later when analyzing data

1. Set temp of water bath or heating block to 25°C
2. Add 220µL assay buffer to all wells.
3. Add 20µL assay buffer to MPO zero standard. 20µL ddH2o to Blank
4. Add 20µL Sample per well
5. Incubate 5 min
6. Add 10µL working H_2O to each well.
7. Incubate 30 min
8. Add 10µL working Catalase to each well
9. Incubate 5 min
10. Add 25µL working TNB to each well and 25µL assay buffer to Blank and mix well

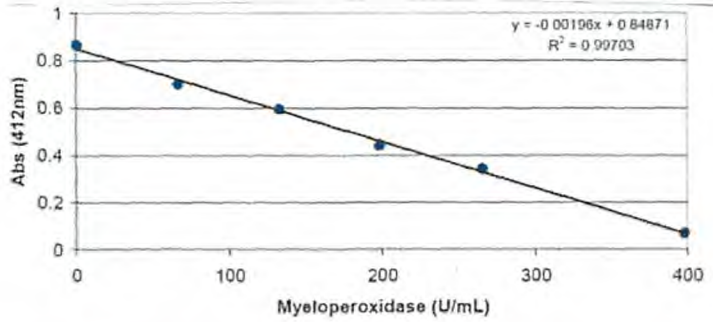
11. Incubate 20 min
12. Read at 412 nm
* If absorbance is lower than 0.06, dilute and repeat.

- Data Analysis

1. Calculate the average absobance at 412 nm for zero MPO samp
2. Calculate the Average for Blank
3. Using the extinction coefficient for TNB of 14100 $M^{-1}cm^{-1}$,
   - using formula to calculate MPO activity

Enzyme Protocol units MPO/ml $= \frac{[(A_{base} + A_{blank} - A_{sample})}{0.0141 \times 2} \times \frac{1140}{80}$

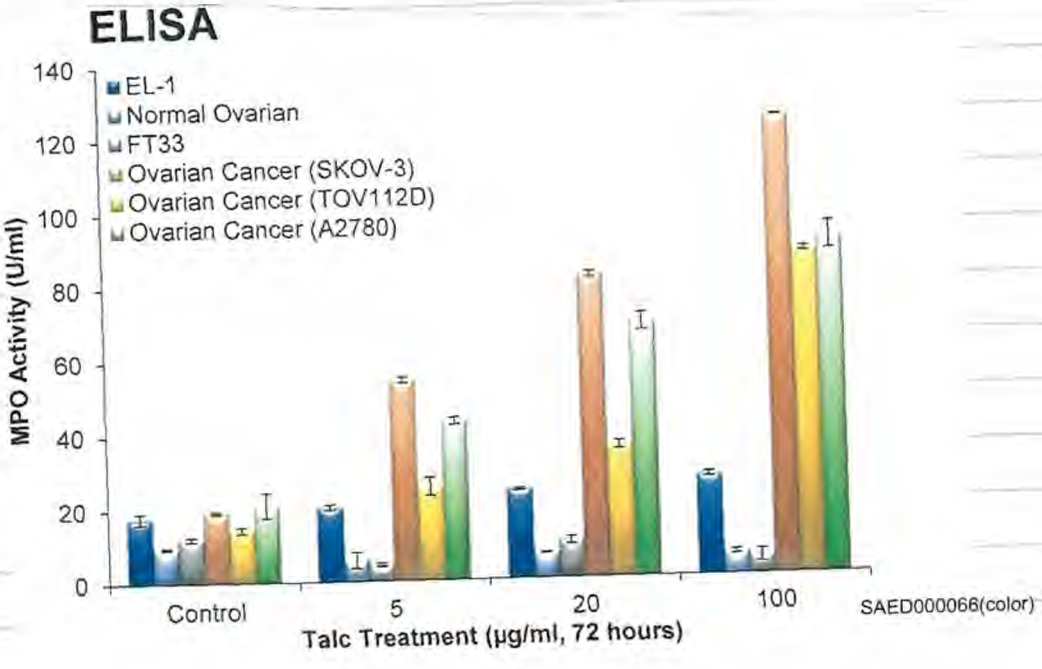
$= 505.3 \times (A_{base} + A_{blank} - A_{sample})$



1.3	PlateFormat	Endpoint	Absorbance	Raw	FALSE	1					
1	2	3	4	5	6	7	8	9	10	11	12
0.0952	0.145	0.223	0.4322	0.4591	0.4369	0.1274	0.1303	0.1224	0.1313	0.1237	0.1207
0.3796	0.3741	0.3151	0.143	0.1267	0.1289	0.1351	0.1559	0.124	0.1253	0.1215	0.1382
0.3701	0.3678	0.4214	0.1254	0.127	0.1248	0.1347	0.1504	0.1304	0.121	0.1275	0.1269
0.4123	0.4128	0.415	0.1395	0.1218	0.1296	0.1243	0.1243	0.1302	0.1331	0.1288	0.1304
0.4279	0.4102	0.4248	0.136	0.1352	0.1449	0.1311	0.1346	0.132	0.1432	0.1483	0.1582
0.4207	0.4258	0.4376	0.137	0.1416	0.1292	0.1301	0.1268	0.134	0.1265	0.1307	0.1287
0.46	0.4589	0.5677	0.1232	0.1293	0.1363	0.134	0.1424	0.1287	0.1469	0.1403	0.1294
0.3904	0.3683	0.3882	0.123	0.1396	0.1211	0.1218	0.1273	0.1237	0.1617	0.1179	0.1176

5/18/2018									
used 5 ug protein				extinction coefficient = 14,100/m*cm					
	OD1	OD2	OD3	Avg Abs	Units MPO/ml	Units MPO/ml	Units MPO/ml	Average	St Dev
MPO BLANK	0.0952	0.145	0.223	0.1201					
MPO zero standard	0.1796	0.1741	0.1151	0.17685					
TOV-112-C	0.2701	0.2678	0.244		13.567305	14.729495	26.755635	14.1484	0.8217924
TOV112-5 ug	0.2523	0.2428	0.245		22.561645	27.361995	26.250335	25.391325	2.5128199
TOV112- 20ug	0.2279	0.2102	0.2248		34.890965	43.834775	36.457395	35.67418	1.1076333
TOV112-100 ug	0.1207	0.1258	0.1376		89.059125	86.482095	80.519555	87.77061	1.8222354
SKOV-3-C	0.26	0.2589	0.2677		18.670835	19.226665	14.780025	18.94875	0.3930312
SKOV-3-5 ug	0.1904	0.2083	0.1882		53.839715	44.794845	54.951375	54.395545	0.7860623
SKOV-3-20 ug	0.1322	0.1591	0.1369		83.248175	69.655605	80.873265	82.06072	1.679315
SKOV-3-100 ug	0.0512	0.0512	0.061		124.177475	124.177475	119.225535	121.70151	3.5015504
A2780-C	0.2449	0.2673	0.2544		26.300865	14.982145	21.500515	20.927842	3.39436
A2780-5 ug	0.2007	0.21	0.2133		48.635125	43.935835	42.268345	45.451735	4.5019933
A2780-20 ug	0.157	0.1567	0.1633		70.716735	70.868325	67.533345	69.12504	1.8832172
A2780-100 ug	0.1087	0.1167	0.1233		95.122725	91.080325	87.745345	91.434035	3.6943386
EL-1-C	0.1105	0.1542	0.1334		94.213185	72.131575	82.641815	88.4275	11.045054
EL-1-5 ug	0.0972	0.0872	0.0968		100.933675	105.986675	101.135795	101.03474	2.8607895
EL-1-20 ug	0.0532	0.0566	0.0611		123.166875	121.448855	119.175005	121.17094	2.0023741
EL-1-100 ug	0.0234	0.0219	0.0311		138.224815	138.982765	134.334005	136.27941	2.4941218

5/19/2018									
used 5 ug protein				extinction coefficient = 14,100/m*cm					
	OD1	OD2	OD3	Avg Abs	Units MPO/ml	Units MPO/ml	Units MPO/ml	Average	St Dev
MPO BLANK	0.0495	0.0528	0.0628	0.0550333					
MPO zero standard	0.027	0.0289	0.02059	0.0254967					
Normal ovarian-C	0.0624	0.0614	0.0619		9.161089	9.666389	9.413739	9.413739	0.25265
Normal Ovarian-Talc 5 ug	0.0734	0.0649	0.069		3.602789	7.897839	5.826109	5.775579	2.1479708
Normal ovarian-Talc 20 ug	0.067	0.0668	0.0675		6.836709	6.937769	6.584059	6.786179	0.1821885
Normal Ovarian-100 ug	0.075	0.0698	0.0681		2.794309	5.421869	6.280879	5.851374	0.6074118
Fallopian-C	0.056	0.0577	0.0575		12.395009	11.535999	11.637059	11.856022	0.4695032
Fallopian-5 ug	0.0696	0.0734	0.0724		5.522929	3.602789	4.108089	4.411269	0.9953256
Fallopian-20ug	0.0546	0.06127	0.0661		13.102429	9.732078	7.291479	10.041995	2.9178454
Fallopian-100 ug	0.0661	0.07128	0.0751		7.291479	4.674025	2.743779	4.9030943	2.2824873



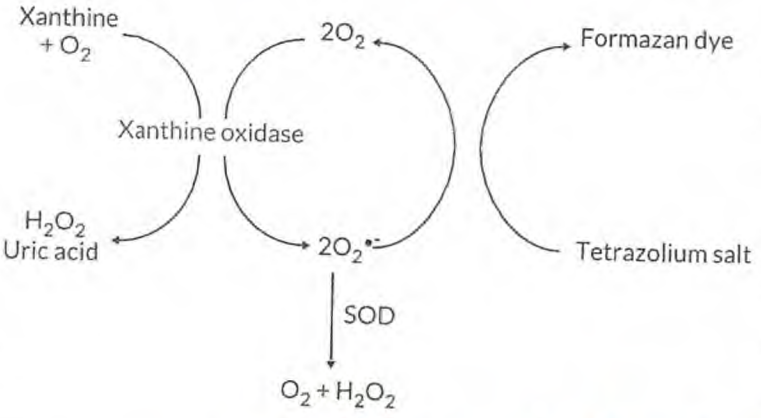
6/19/2018 Superoxide Dismutase Assay Kit

Caymanchem. Cat#706002

Superoxide dismutases (SODs)

$$2O_2^{\bullet-} + 2H^+ + SOD \rightarrow H_2O_2 + O_2$$

- This kit utilizes a tetrazolium salt for detection of superoxide radicals generated by xanthine oxidase and hypoxanthine



Scheme of Superoxide Dismutase Assay

- The SOD assay measures all three types of SOD (Cu/Zn, Mn, and FeSOD)
- One unit of SOD is defined as amount of enzyme needed to exhibit 50% dismutation of the superoxide radical.

Reagent Preparation

- Assay Buffer (10X)
  - Dilute 3 ml of Assay Buffer with 27 ml of HPLC-grade water. (1X)
  - Store at 4°C. stable for 2 months.

- Sample Buffer (10X)
  - Dilute 2ml of Sample Buffer with 18 mL of HPLC-water (1X, 50mM Tris-HCl)
  - Store at 4°C stable for 6 months

SAED000067(color)

- Radical Detector
  - 250µl of tetrazolium salt solution
  - Prior to use. 50µl of solution + 19.95ml diluted Assay Buffer.
  - ※ Cover with tin foil
  - Stable for 2 months. enough for 96 well
  - Store unused at -20°C

- SOD Standard
  - Contain 100µl of bovine erythrocyte SOD(Cu/Zn)
  - Store the thawed enzyme on ice
  - Store at -20°C, stable two freeze/thaw cycles.

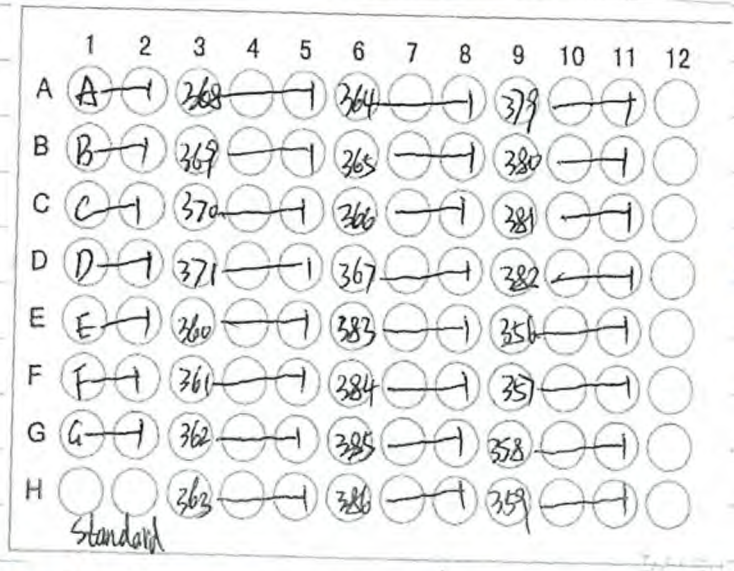
- Xanthine Oxidase
  - Contain 150µl of Xanthine Oxidase.
  - Prior to use, thaw one vial and transfer 50µl of supplied enzyme with 1.95ml of diluted ~~of~~ sample Buffer
  - This diluted enzyme is stable for one hour
  - ※ Do not refreeze the thawed enzyme

Sample preparation

- Cell lysate see pg 53
- Collect cells by centrifugation at 1000-2000g, 10 min, 4°C
- ※ For adherent cells, use a rubber policeman.
- Homogenize or sonicate the cell pellet in cold 20mM Hepes.
- Centrifuge at 1500xg, 5 min. 4°C
- Remove the supernatant for assay and store on ice
- freeze sample at -8°C
- Stable for two months.

Assay protocal

- Plate set up (as following sheet)



Sample plate format

A-G : Standards

* Final volume is 230μl /well
* Assay temperature is 25°C
* Read at 440~460 nm.

- Standard Preparation
  - Dilute 20μl of SOD standard with 1.98 ml Sample buffer (dilute)
  - Take 7 clean glass test tubes and mark A-G
  - Add amount of SOD stock and Sample Buffer to each tubes. as below

Tube	SOD Stock (μl)	Sample Buffer (μl)	Final SOD Activity (U/ml) in Well
A	0	1,000	0
B	20	980	0.005
C	40	960	0.010
D	80	920	0.020
E	120	880	0.030
F	160	840	0.040
G	200	800	0.050

Table 1. Superoxide Dismutase standards

Performing the Assay

1. SOD Standard Wells
   - add 200µl of diluted Radical Detector and 10µl of standard

2. Sample wells
   - add 200µl of the diluted Radical Detector and 10µl of samples

3. Initiate the reactions by adding 20µl of diluted Xanthine Oxidase to all the wells.
   - ※ Make sure to note the precise time you started
   - ※ Add Xanthine Oxidase as quickly as possible

4. Carefully shaker for 30 minutes at room temp

5. Read at 440 ~ 460 nm

Calculation

- Calculate the average absorbance of each standard and sample
- Divide standard A's absorbance by itself and divide standard A's absorbance by all the other standards and samples absorbances to yield the Linearized Rate
- Plot the Linearized SOD ~~acti~~ standard rate as function of final SOD Activity (U/ml)
- Calculate the SOD activity of the samples using the equation obtained from the linear regression of standard curve substituting the Linearized rate for each sample

$$\text{SOD (U/ml)} = \left[\left(\frac{\text{sample LR - y-intercept}}{\text{slope}}\right) \times \frac{0.23\ \text{ml}}{0.01\ \text{ml}}\right] \times \text{sample dilution}$$

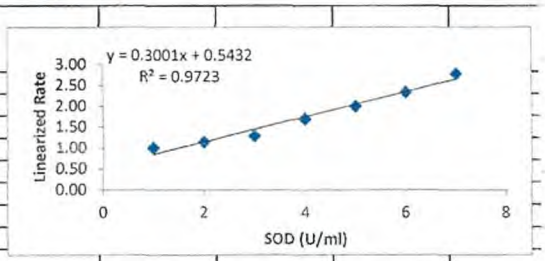
NOTE: 0.23/0.01 is a factor for converting from U/ml in well to U/ml in 10 µl added to 230 µl well volume

X The dynamic range of kit is 0.005 - 0.050 units/ml SOD

- One unit is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. ~~SOD~~

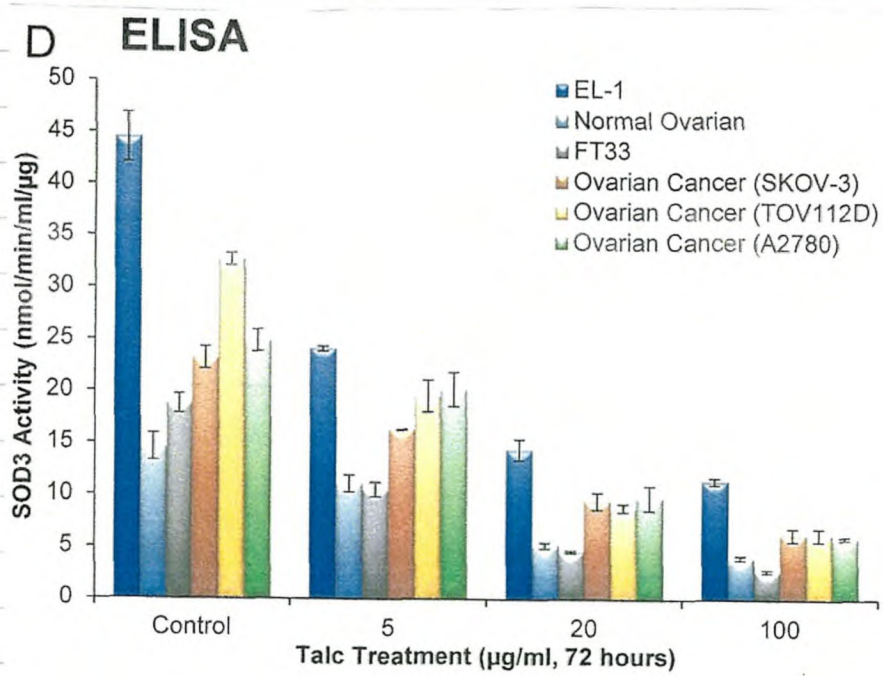
Standard Curve

SOD Activity (U/ml)	Standard	Raw 1	Raw 2	Average	Linearized rate (LR)
0	A	0.4759	0.4618	0.46885	1.00
0.025	B	0.4385	0.3845	0.4115	1.14
0.05	C	0.3786	0.353	0.3658	1.28
0.1	D	0.2872	0.2671	0.2772	1.69
0.15	E	0.2373	0.2336	0.2355	1.99
0.2	F	0.203	0.1988	0.2009	2.33
0.25	G	0.1747	0.164	0.1694	2.77



0.7 ug Protein

6/19/2013		OD 1	OD 2	OD 3	LR 1	LR 2	LR 3	U/ml	U/ml	U/ml	Average	SD
BLANK		0.3138	0.3167	0.3187								
	A2780-C	0.0405	0.0533	0.0562	11.576543	8.796435	8.342527	34.709321	25.615603	24.13087	24.87	1.05
	A2780-5 ug	0.0744	0.0669	0.0582	6.301747	7.008221	8.055842	17.455491	19.766364	23.193126	20.14	2.89
	A2780-20 ug	0.1022	0.1235	0.1426	4.587573	3.796356	3.287868	11.848437	9.2603704	7.5971084	9.72	1.83
	A2780-100 ug	0.1722	0.1688	0.1685	2.722706	2.777547	2.782493	5.7484664	5.9278518	5.9440275	5.94	0.14
	SKOV-3-C	0.0498	0.0600	0.0566	9.414659	7.814167	8.283569	27.637808	22.402607	23.93802	23.17	1.09
	SKOV-3-5 ug	0.0786	0.0678	0.0790	5.965013	6.915192	5.934810	16.354034	19.462065	16.255242	18.30	0.07
	SKOV-3-20 ug	0.1036	0.1165	0.1276	4.525579	4.024464	3.674373	11.645655	10.006508	8.8613639	9.43	0.81
	SKOV-3-100 ug	0.1651	0.1556	0.1711	2.8397941	3.0131748	2.7402104	6.1314604	6.6985879	5.8057227	6.25	0.63
	TOV-112-C	0.0433	0.0359	0.0433	10.827945	13.059889	10.827945	32.260659	39.561336	32.260659	35.91	5.16
	TOV112-5 ug	0.0683	0.0611	0.0757	6.8645681	7.6734861	6.1935271	19.296475	21.942442	17.101504	19.52	3.42
	TOV112- 20ug	0.1143	0.1253	0.1311	4.1019248	3.7418196	3.5762777	10.259883	9.0819813	8.5404943	8.81	0.38
	TOV112-100 ug	0.1654	0.1559	0.1711	2.8346433	3.0073765	2.7402104	6.1146122	6.6796217	5.8057227	6.24	0.62
	Normal ovarian-C	0.0855	0.0954	0.0789	5.4836257	4.9145702	5.9423321	14.77942	12.918042	16.279846	14.60	2.38
	Normal Ovarian-Talc 5 ug	0.1130	0.1035	0.1120	4.149115	4.5299517	4.1861607	10.414242	11.659957	10.535419	11.10	0.80
	Normal ovarian- Talc 20 ug	0.1774	0.1792	0.1883	2.6428974	2.6163504	2.4899097	5.4874124	5.4005774	4.9869905	5.19	0.29
	Normal Ovarian-100 ug	0.2003	0.2100	0.2187	2.3407389	2.232619	2.1438043	4.4990535	4.145394	3.8548814	4.00	0.21
	Fallopian-C	0.0773	0.0679	0.0722	6.0653299	6.9050074	6.492868	16.682171	19.428752	18.080646	18.75	0.95
	Fallopian-5 ug	0.1156	0.1162	0.1083	4.0557958	4.0348537	4.3291782	10.108996	10.040494	11.003228	10.52	0.68
	Fallopian-20ug	0.1896	0.1964	0.2005	2.4728376	2.38722	2.338404	4.9311475	4.6510928	4.491416	4.57	0.11
	Fallopian-100 ug	0.2451	0.2561	0.2650	1.9128927	1.8307302	1.7692453	3.0995708	2.8308176	2.6297008	2.73	0.14
	EL-1-C	0.0334	0.0411	0.0268	14.037425	11.407543	17.494403	42.758854	34.156521	54.06661	44.11	14.08
	EL-1-5 ug	0.0567	0.0765	0.0567	8.2689594	6.1287582	8.2689594	23.890232	16.889645	23.890232	20.39	4.95
	EL-1-20 ug	0.0886	0.0841	0.0912	5.2917607	5.5749108	5.1408991	14.151831	15.078013	13.658363	14.37	1.00
	EL-1-100 ug	0.1005	0.1042	0.1076	4.6651741	4.4995202	4.357342	12.102269	11.560416	11.095352	11.33	0.33



SAED000073(color)

6/21/2018

Caspase-3 Colorimetric Assay
R&D systems, Cat# BF3100

Reagents provided & storage conditions

REAGENT	DESCRIPTION	STORAGE OF OPENED MATERIAL
DEVD-pNA Substrate	500 µL of 4 mM DEVD substrate peptide conjugated to p-nitroaniline **(protect from light).**	Store at ≤ -20 °C for up to 6 months after initial use. Avoid repeated freeze-thaw cycles.
DTT	400 µL of a 1 M solution of dithiothreitol (DTT).	
Lysis Buffer	100 mL of Lysis Buffer.	May be stored for up to 6 months at 2-8 °C.
Reaction Buffer 3	4 vials (2.0 mL/vial) of 2X Reaction Buffer 3.	
Dilution Buffer	100 mL of Dilution Buffer.	

* Store the unopend kit at -20°C in a manual defrost freezer

- This kit use to determine the increased enzymatic activity of Caspase-3 class of proteases in apoptotic cells by colorimetric reaction.

- Caspase-3 known as CPP-32, Yama or Apopain, is an intracellular cysteine protease that exists as a proenzyme, becoming activated during the cascade of events associated with apoptosis.

- The presence of caspase-3 in cells of different lineages suggests that caspase-3 is a key enzyme required for the execution of apoptosis.

- The cleavage of peptide by the caspase releases the chromophore pNA, which can be quantitated spectrophotometrically at wavelength of 405nm

- The level of caspase enzymatic activity in the cell lysate is directly proportional to the color reaction.

Sample preparation:

1. Collect cells, 250×g, 10 minutes
- Add 25µL of cold Lysis Buffer per 1×10^6 cells.
- Seed 10^6 cells per dish

SAED000074(color)

2. The cell lysate is incubated on ice for 10 minutes
   - centrifuged at 10000xg for 1 minute.
   - Transfer the supernate to a new tube and on ice

3. The enzymatic reation for caspase activity is best carried out in a 96 well flat bottom microplate

4. Each reaction requires 50µl of cell lysate.

5. Each reaction also requires 50µl of 2X Reaction Buffer 3.
   - Prior to using the 2X Reaction Buffer 3
   - Add 10µL of fresh DTT stock per 1mL of 2X Reaction 3

6. To each reaction well add 5µl of caspase-3 colorimetric substrate

7. ~~To~~ Incubate the plate 37°C, 1~2hours.

8. Read the plate on a microplate reader using a wavelength of 405nm

9. Additional control that should be included in this assay
   - no cell lysate and no substrate.
   - The total reaction volume must be kept constant and therefore distilled can be used to replace the volume by cell lysate.

10. For comparative analysis, the above assay should be repeated with non-induced cells.

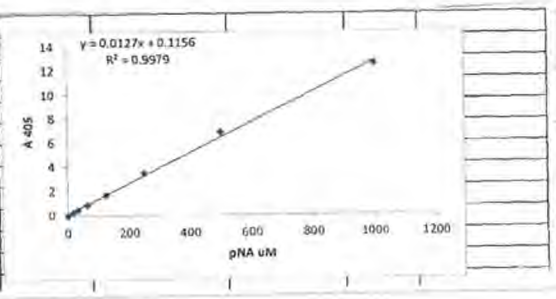
Standard : Dilute in 1X Assay Buffer
- 1:2 dilution series 120µl of standard + 120 µl buffer

S1	1mM	S5	62.5µM
S2	500µM	S6	31.3µM
S3	250µM	S7	15.6µM
S4	125µM	S8	1µM

SAED000075(color)

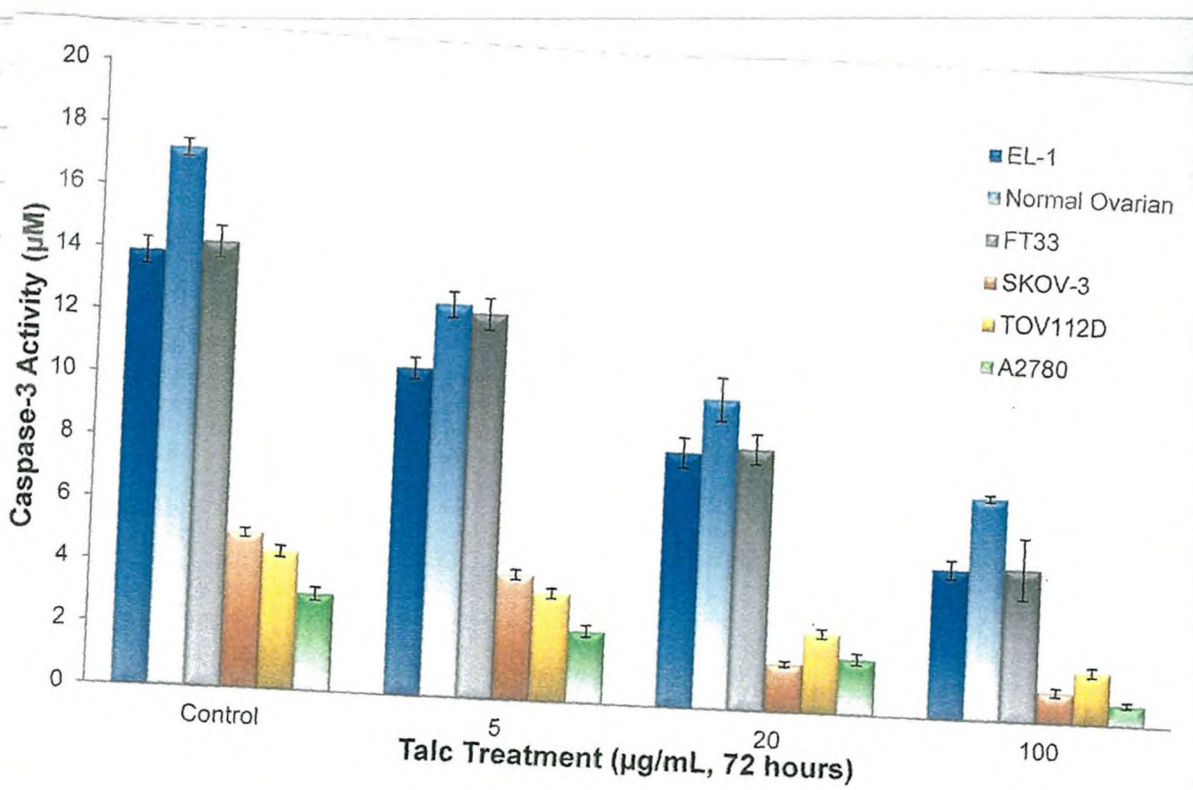
	1	2	3	4	5	6	7	8	9	10	11	12
A	A			368			364			379		
B	B			369			365			380		
C	C			370			366			381		
D	D			371			367			382		
E	E			360			383			356		
F	F			361			384			357		
G	G			362			385			358		
H				363			386			359		

Standard	Raw1	Raw2	Raw3	Ave. Abs.	[Csps-3](uM)
1	0	0	0	0	0
2	0.203	0.137	0.208	0.206	15.625
3	0.419	0.433	0.487	0.446	31.25
4	0.844	0.848	0.891	0.861	62.5
5	1.642	1.671	1.714	1.676	125
6	3.455	3.512	3.497	3.488	250
7	6.716	6.778	7.063	6.852	500
8	12.503	12.604	18.265	12.554	1000
				y = 0.0127x + 0.1156	



Protein

Caspase-3 assay										
100 ug protein										
Sample	Raw1	Raw2	Raw3	Average	[Csps-3]-1	[Csps-3]-2	[Csps-3]-3	[Csps-3] (uM)	SE	
A2780-C	0.198	0.192	0.189	0.194	6.488	6.016	5.780	3.105	0.361	
A2780-5 ug	0.177	0.172	0.169	0.173	4.835	4.441	4.205	2.307	0.318	
A2780-20 ug	0.155	0.159	0.151	0.153	3.102	3.417	2.787	1.785	0.315	
A2780-100 ug	0.132	0.131	0.135	0.134	1.291	1.213	1.528	0.673	0.164	
SKOV-3-C	0.233	0.239	0.237	0.235	9.244	9.717	9.559	4.976	0.241	
SKOV-3-5 ug	0.211	0.215	0.218	0.215	7.512	7.827	8.063	4.021	0.277	
SKOV-3-20 ug	0.155	0.153	0.157	0.156	3.102	2.945	3.260	1.550	0.157	
SKOV-3-100 ug	0.133	0.139	0.137	0.135	1.370	1.843	1.685	0.989	0.241	
TOV-112-C	0.220	0.225	0.228	0.224	8.220	8.614	8.850	4.419	0.318	
TOV112-5 ug	0.198	0.201	0.194	0.196	6.488	6.724	6.173	3.460	0.277	
TOV112- 20ug	0.177	0.178	0.183	0.180	4.850	4.921	5.315	2.551	0.250	
TOV112-100 ug	0.155	0.156	0.150	0.152	3.110	3.197	2.685	1.675	0.274	
Normal ovarian-C	0.551	0.546	0.558	0.555	34.283	33.890	34.835	17.222	0.475	
Normal Ovarian-Talc 5 ug	0.435	0.429	0.447	0.441	25.150	24.677	26.094	12.559	0.722	
Normal ovarian- Talc 20 ug	0.333	0.363	0.344	0.339	17.118	19.480	17.984	9.909	1.195	
Normal Ovarian-100 ug	0.288	0.292	0.288	0.288	13.583	13.913	13.567	7.101	0.196	
Fallopian-C	0.488	0.471	0.492	0.490	29.323	27.984	29.638	14.237	0.878	
Fallopian-5 ug	0.411	0.423	0.401	0.406	23.260	24.213	22.472	12.309	0.871	
Fallopian-20ug	0.312	0.324	0.333	0.323	15.472	16.417	17.118	8.370	0.826	
Fallopian-100 ug	0.254	0.237	0.211	0.219	10.898	9.535	7.512	4.877	1.704	
EL-1-C	0.445	0.463	0.449	0.447	25.937	27.354	26.252	13.901	0.744	
EL-1-5 ug	0.389	0.377	0.391	0.390	21.528	20.583	21.685	10.486	0.596	
EL-1-20 ug	0.311	0.319	0.298	0.305	15.386	16.016	14.362	8.160	0.835	
EL-1-100 ug	0.221	0.234	0.228	0.225	8.299	9.323	8.850	4.774	0.512	



SAED000077(color)

6/29/2018 SNP Genotyping Assay

SNP

(Applied Biosystems, Carlsbad. CA)

- SNP to be examined in cell pellets
- DNA was isolated utilizing the EZ1 DNA Tissue Kit (Qiagen) for EOC Cells according the manufacter's protocols
- The TaqMan SNP Genotyping Assay set were used to genotype the SNP.

A	Gene (rs number)				
	CAT (rs769217)	NOS2 (rs2297518)	GSR (rs8190955)	GPX1 (rs3448)	SOD3 (rs2536512)
MAF	0.123	0.173	0.191	0.176	0.476
SNP	C-262T	C2087T	G201T	C-1040T	A377T
Chromosome Location	11p13	17q11.2	8p12	3q21.31	4p15.2
Amino Acid Switch	Isoleucine to Threonine	Serine to Leucine	Unknown	Unknown	Alanine to threonine
Effect on Activity	Decrease	Increase	Unknown	Unknown	Decrease

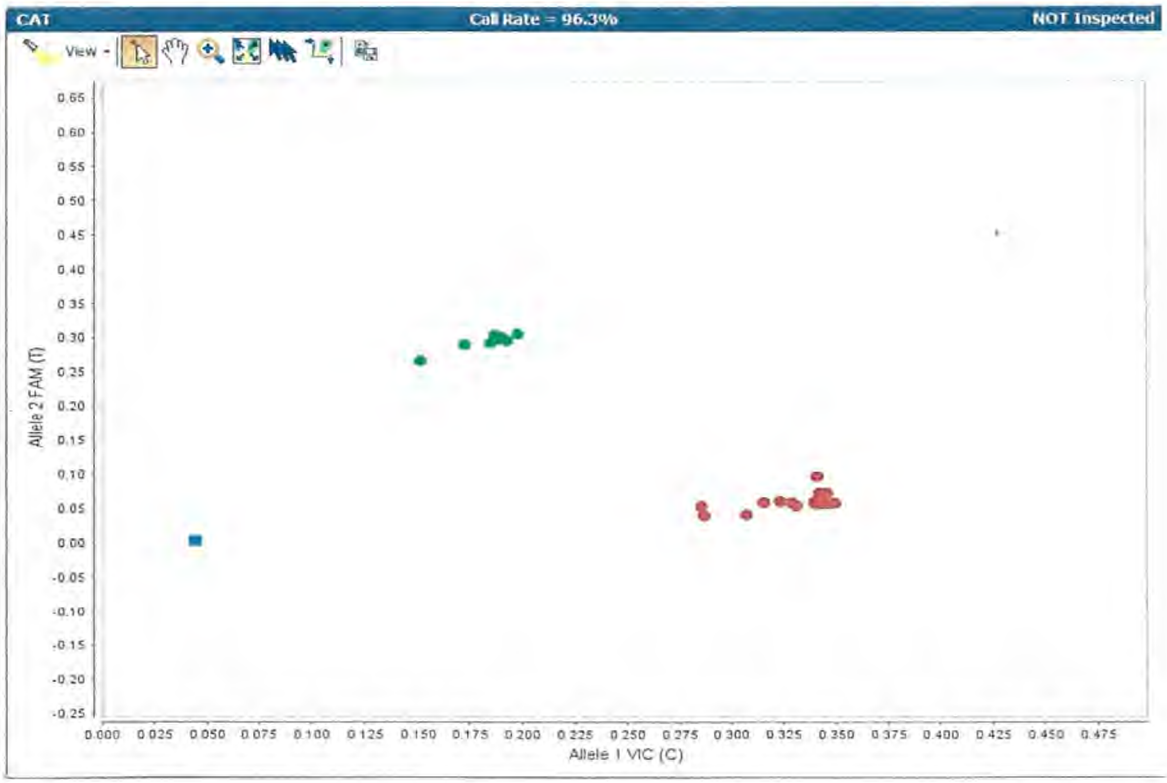
- The TaqMan SNP Genotyping Assay Set were used to genotype the SNPs
  - NCBI dbSNP genome ~~Technology Cen~~ build 37, MAF source 1000 genomes
- The Applied Genomics Technology Center performed these assay
  - AGTC, Wayne State University, Detroit, MI
- Analysis was done utilizing the QuanStudio™ 12K Flex Real-time PCR System.

SAED000078(color)

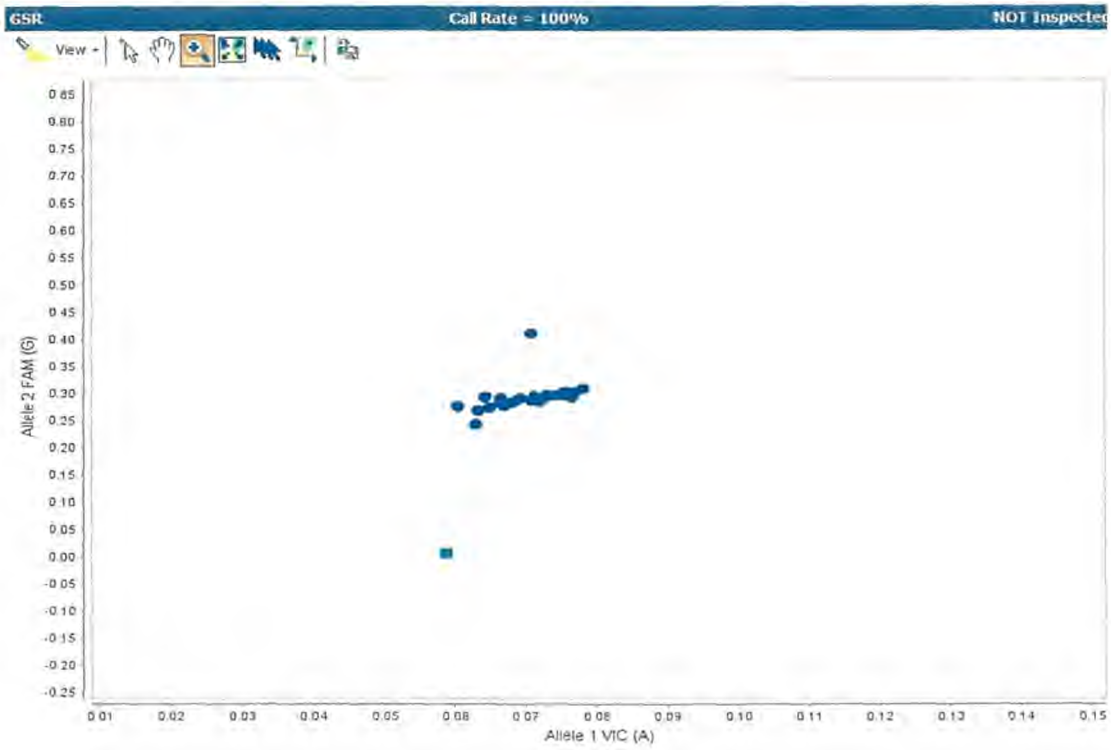
Exported By : GUEST
Export Date : 07/11/2018 14:11:18 EDT
Study Name : Untitled
Experiment Type : Endpoint
Instrument Type : QuantStudio™ 12K Flex Real-Time PCR System
Software Version Number : 1.4.0
Creation Date : 07/11/2018 13:20:55 EDT
Created By : GUEST
Last Modified Date : 07/11/2018 14:10:49 EDT
Last Modified By : GUEST
Template File Name : N/A
Template Originating Study Name : N/A
Template Creation Date : N/A
Template Created By User ID : N/A
Template Software Version Number : N/A

	Assay ID	Assay Nam	Population	Allele 1 Fre	Allele 2 Fre	1/1 Freq	1/2 Freq	2/2 Freq	Chi-Squared	P-Value
5	SNP Assay	SOD3	All	0%	0%	0%	0%	0%	0	1
4	SNP Assay	NOS2	All	31.50%	68.50%	14.80%	33.30%	51.90%	1.396	0.237
3	SNP Assay	GPX1	All	100%	0%	100%	0%	0%	0	1
2	SNP Assay	GSR	All	0%	100%	0%	0%	100%	0	1
1	SNP Assay	CAT	All	84.60%	15.40%	69.20%	30.80%	0%	0.86	0.354
	SNP Assay	CYBA	All	41.70%	58.30%	12.50%	58.30%	29.20%	0.96	0.327

Assay Name	Assay ID	NCBI SNP Ref.	Sample ID	Call	Allele1 (C) Amp Score	Allele2 (T) Amp Score	Well	Experiment Name
CAT	SNP Assay 5	rs769217	A2780-C	C/C	0.859261	0.000000	I01	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	SKOV-C	C/C	0.868249	0.000000	I03	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	TOV112-C	C/C	0.867341	0.000000	I05	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	HOSPIC-C	C/C	0.875622	0.000000	I07	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	FT33-C	C/C	0.871144	0.000000	I09	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	NOC-C	C/C	0.876471	0.000000	I11	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	A2780-T	C/C	0.877593	0.000000	I13	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	SkOV-T	C/C	0.872718	0.000000	I15	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	TOV112-T	C/T	0.658010	0.869565	I17	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	HOSPIC-T	C/T	0.655459	0.868229	I19	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	FT33-T	C/T	0.650990	0.864536	I21	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	NOC-T	C/T	0.612055	0.850921	I23	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	A2780-C	C/C	0.841331	0.000000	I02	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	SKOV-C	C/C	0.860892	0.000000	I04	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	TOV112-C	C/C	0.876584	0.000000	I06	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	HOSPIC-C	C/C	0.874654	0.000000	I08	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	FT33-C	C/C	0.877596	0.000000	I10	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	NOC-C	C/C	0.874607	0.000000	I12	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	A2780-T	C/C	0.867310	0.000000	I14	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	SkOV-T	C/C	0.871008	0.000000	I16	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	TOV112-T	C/T	0.656320	0.877184	I18	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	HOSPIC-T	C/T	0.649022	0.863312	I20	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	FT33-T	C/T	0.648668	0.867816	I22	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	NOC-T	C/T	0.629139	0.864298	I24	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	CEPH	C/C	0.838256	0.000000	N04	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	NTC	N/A	0.000000	0.000000	N06	2018-07-11_GS-997.eds
CAT	SNP Assay 5	rs769217	SJL	C/C	0.835160	0.000000	N02	2018-07-11_GS-997.eds



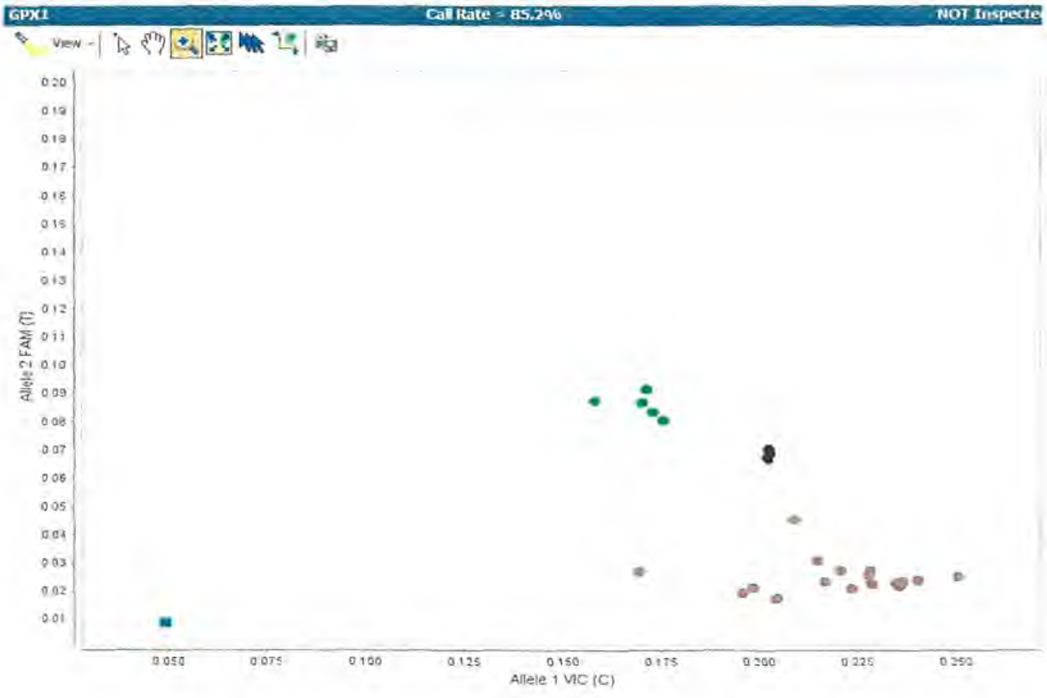
Assay Nam	Assay ID	NCBI SNP Ref.	Sample ID	Call	Allele1 (A) Amp Score	Allele2 (G) Amp Score	Well	Experiment Name
GSR	C__25472285_20	rs8190955	A2780-C	G/G	0.000000	0.893638	G01	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	SKOV-C	G/G	0.000000	0.897784	G03	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	TOV112-C	G/G	0.000000	0.900424	G05	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	HOSPIC-C	G/G	0.000000	0.903515	G07	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	FT33-C	G/G	0.000000	0.899146	G09	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	NOC-C	G/G	0.000000	0.903931	G11	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	A2780-T	G/G	0.000000	0.907440	G13	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	SkOV-T	G/G	0.000000	0.904642	G15	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	TOV112-T	G/G	0.000000	0.903133	G17	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	HOSPIC-T	G/G	0.000000	0.898479	G19	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	FT33-T	G/G	0.000000	0.889356	G21	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	NOC-T	G/G	0.000000	0.865288	G23	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	A2780-C	G/G	0.000000	0.777331	H01	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	SKOV-C	G/G	0.000000	0.890199	G02	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	TOV112-C	G/G	0.000000	0.894693	G04	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	HOSPIC-C	G/G	0.000000	0.907142	G06	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	FT33-C	G/G	0.000000	0.910245	G08	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	NOC-C	G/G	0.000000	0.906755	G10	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	A2780-T	G/G	0.000000	0.905957	G12	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	SkOV-T	G/G	0.000000	0.898448	G14	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	TOV112-T	G/G	0.000000	0.900353	G16	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	HOSPIC-T	G/G	0.000000	0.900184	G18	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	FT33-T	G/G	0.000000	0.600299	G20	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	NOC-T	G/G	0.000000	0.889474	G22	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	NOC-T	G/G	0.000000	0.888737	G24	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	CEPH	G/G	0.000000	0.891366	M04	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	NTC	N/A	0.000000	0.000000	M06	2018-07-11_GS-997.eds
GSR	C__25472285_20	rs8190955	SJL	G/G	0.000000	0.894044	M02	2018-07-11_GS-997.eds



2

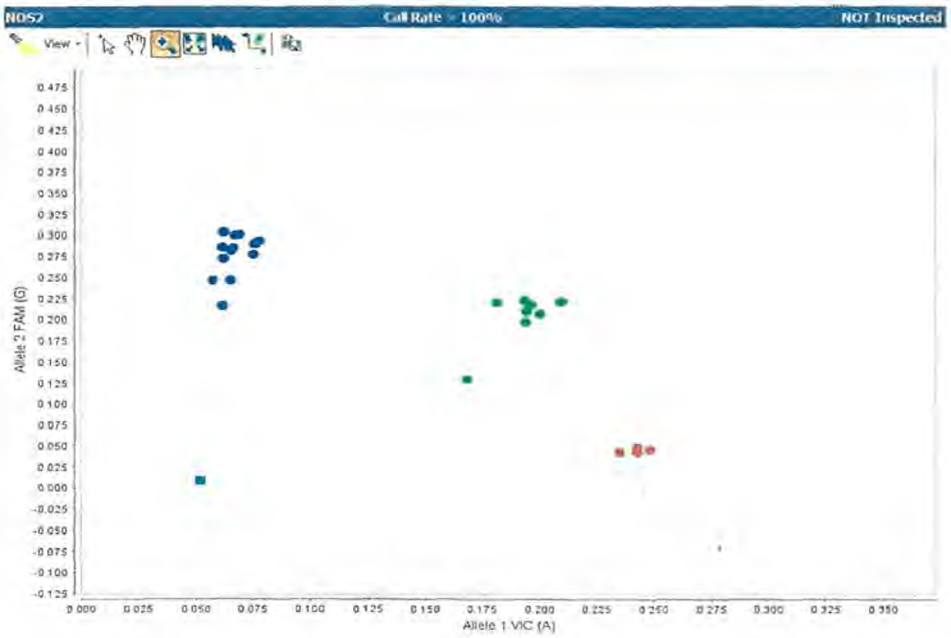
SAED000081(color)

Assay Name	Assay ID	NCBI SNP Ref.	Sample ID	Call	Allele1 (C) Amp Score	Allele2 (T) Amp Score	Experiment Name
GPX1	C___8762057_10	rs3448	A2780-T	C/C	0.712653	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	SKOV-T	C/C	0.705939	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	FT33-T	C/C	0.732661	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	NOC-T	C/C	0.741459	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	A2780-C	UND	0.673660	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	SKOV-C	UND	0.671252	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	FT33-C	UND	0.659262	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	NOC-C	UND	0.672411	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	A2780-T	C/C	0.730852	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	SKOV-T	C/C	0.724943	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	FT33-T	C/C	0.717511	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	NOC-T	C/C	0.701899	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	EL-1	C/C	0.607089	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	A2780-C	C/T	0.626308	0.543334	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	SKOV-C	C/T	0.621549	0.554203	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	FT33-C	C/T	0.603927	0.530074	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	NOC-C	C/T	0.608042	0.532584	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	A2780-T	C/C	0.730550	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	SKOV-T	C/C	0.651890	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	FT33-T	C/C	0.744586	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	NOC-T	C/C	0.727160	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	A2780-T	C/C	0.731175	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	SKOV-T	C/C	0.714878	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	FT33-T	C/C	0.724256	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	NOC-T	C/C	0.685770	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	CEPH	C/T	0.592382	0.526196	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	NTC	N/A	0.000000	0.000000	2018-07-11_GS-997.eds
GPX1	C___8762057_10	rs3448	SJL	C/C	0.701809	0.000000	2018-07-11_GS-997.eds



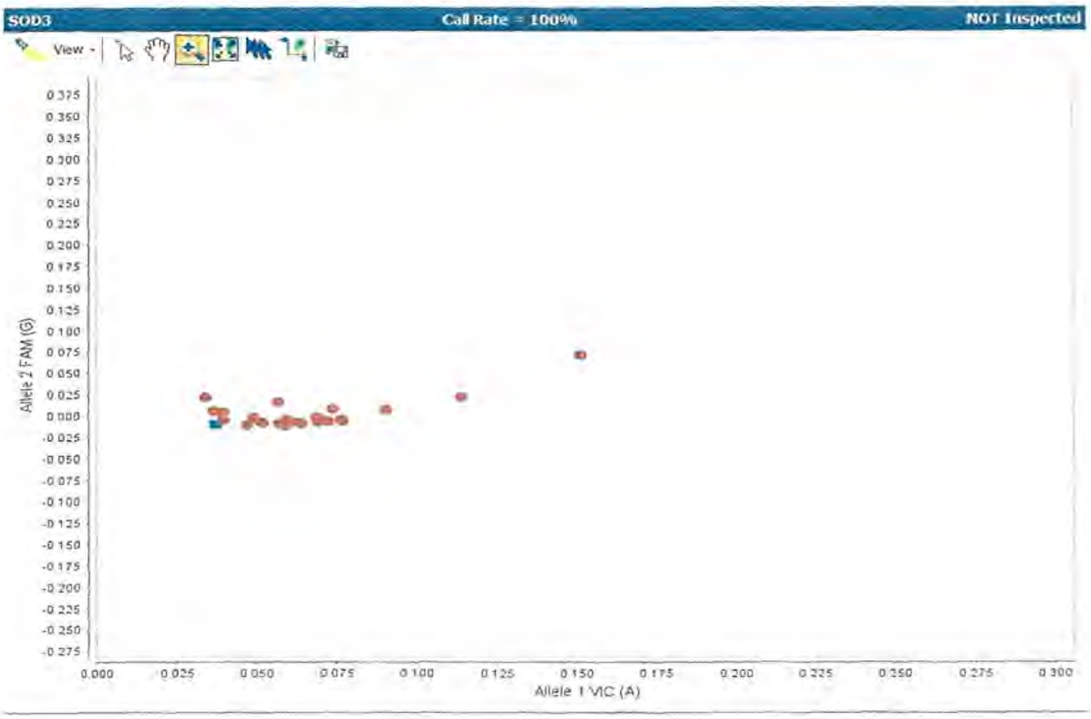
3

Assay Name	Assay ID	NCBI SNP Ref.	Sample ID	Call	Allele1 (A) Amp Score	Allele2 (G) Amp Score	Well	Experiment Name
NOS2	C__11889257_10	rs2297518	A2780-C	G/G	0	0.878929	C01	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	TOV112-C	G/G	0	0.873627	C03	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	SKOV-C	G/G	0	0.869711	C05	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	HOSPIC-C	G/G	0	0.794009	C07	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	FT33-C	G/G	0	0.88025	C09	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	NOC-C	G/G	0	0.881837	C11	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	A2780-C	G/G	0	0.877297	C13	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	TOV112-C	G/G	0	0.866705	C15	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	SKOV-C	G/G	0	0.879188	C17	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	HOSPIC-C	G/G	0	0.881639	C19	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	FT33-C	G/G	0	0.870062	C21	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	NOC-C	G/G	0	0.818528	C23	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	A2780-T	G/G	0	0.740608	D01	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	TOV112-T	A/G	0.685417	0.770813	C02	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	SKOV-T	A/G	0.700276	0.780029	C04	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	HOSPIC-T	A/G	0.604773	0.599274	C06	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	FT33-T	A/G	0.696461	0.764702	C08	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	NOC-T	A/G	0.685289	0.770144	C10	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	A2780-T	A/G	0.700586	0.782077	C12	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	TOV112-T	A/G	0.709069	0.779647	C14	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	SKOV-T	A/G	0.691319	0.789883	C16	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	HOSPIC-T	A/A	0.782495	0	C18	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	FT33-T	A/A	0.78802	0	C20	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	NOC-T	A/A	0.790621	0	C22	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	NOC-T	A/A	0.778243	0	C24	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	CEPH	G/G	0.000000	0.870160	N03	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	NTC	N/A	0.000000	0.000000	N05	2018-07-11_GS-997.eds
NOS2	C__11889257_10	rs2297518	SJL	A/G	0.666694	0.761451	N01	2018-07-11_GS-997.eds



4

Assay Name	Assay ID	NCBI SNP Ref.	Sample ID	Call	Allele1 (A) Amp Score	Allele2 (G) Amp Score	Experiment Name
SOD3	C___2668728_10	rs2536512	A2780-C	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	TOV112-C	A/A	0.605730	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	SKOV-C	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	HOSPIC-C	A/A	0.532156	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	FT33-C	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	NOC-C	A/A	0.576449	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	A2780-C	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	TOV112-C	A/A	0.521027	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	SKOV-C	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	HOSPIC-C	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	FT33-C	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	NOC-C	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	A2780-T	A/A	0.525351	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	TOV112-T	A/A	0.524933	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	SKOV-T	A/A	0.513045	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	HOSPIC-T	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	FT33-T	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	NOC-T	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	A2780-T	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	TOV112-T	A/A	0.533845	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	SKOV-T	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	HOSPIC-T	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	FT33-T	A/A	0.532481	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	NOC-T	A/A	0.526249	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	NOC-T	A/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	CEPH	A/A	0.622530	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	NTC	N/A	0.000000	0.000000	2018-07-11_GS-997.eds
SOD3	C___2668728_10	rs2536512	SJL	A/A	0.682456	0.559216	2018-07-11_GS-997.eds



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	Gene (rs number)				
Cell Lines	***CAT* (rs769217)**	***NOS2* (rs2297518)**	***GSR* (rs8190955)**	***GPX1* (rs3448)**	***SOD3* (rs2536512)**
A2780- Control	C/C	C/C	G/G	**C/T**	A/A
A2780- Talc	C/C	C/C	G/G	C/C	A/A
SKOV-3- Control	C/C	C/C	G/G	**C/T**	A/A
SKOV-3- Talc	C/C	T/T	G/G	C/C	A/A
TOV112D- Control	C/C	C/C	G/G	**C/T**	A/A
TOV112D-Talc	**C/T**	C/C	G/G	C/C	A/A
HOSEpiC- Control	C/C	C/C	G/G	**C/T**	A/A
HOSEpiC- Talc	**C/T**	**T/T**	G/G	**C/T**	A/A
FT33- Control	C/C	C/C	G/G	**C/T**	A/A
FT33- Talc	**C/T**	**T/T**	G/G	C/C	A/A
Normal Ovarian-Control	C/C	C/C	G/G	**C/T**	A/A
Normal Ovarian-Talc	**C/T**	**T/T**	G/G	C/C	A/A

MTT Cell Proliferation Assay

(Trevigen Gaithersburg, MD)
Cat#4890-25K

MTT Sig He

9/4/2018

- Seeded cells 8000 cells/well
- Count cells using the hemacytometer

96 wells Plate design

	1	2	3	4	5	6	7	8	9	10	11	12
A	A2780 Unt			EL-1Unt								
B	A2780 100ug/ml			EL-1 100ug/ml								
C												
D	SKOV-3 Unt			TOV112 Unt								
E	SKOV-3 100ug/ml			TOV112 100ug/ml								
F												
G	Normal ovarian Unt			FT33 Unt								
H	Normal ovarian 100ug/ml			FT33 100ug/ml								

9/5/2018

- Treat cells with talc

$X \cdot 10^4 \mu g/ml = (5ml)(100\mu g/ml) \Rightarrow X = 50\mu l$

9/6/2018

- After 24 hours treatment
- Add 10μl MTT reagent to each well

- Incubate 2 hours in 37°C incubator
  - ✱ For normal cells, incubate more than 2 hours.
  - Check under microscope to make sure has fromanza.

- Next, add Second reagent (SDS-HCl Detergent Reagent)
  - 100μl per well

- Incubate 2~4 hours in 37°C incubator

- Detect at 570 nm

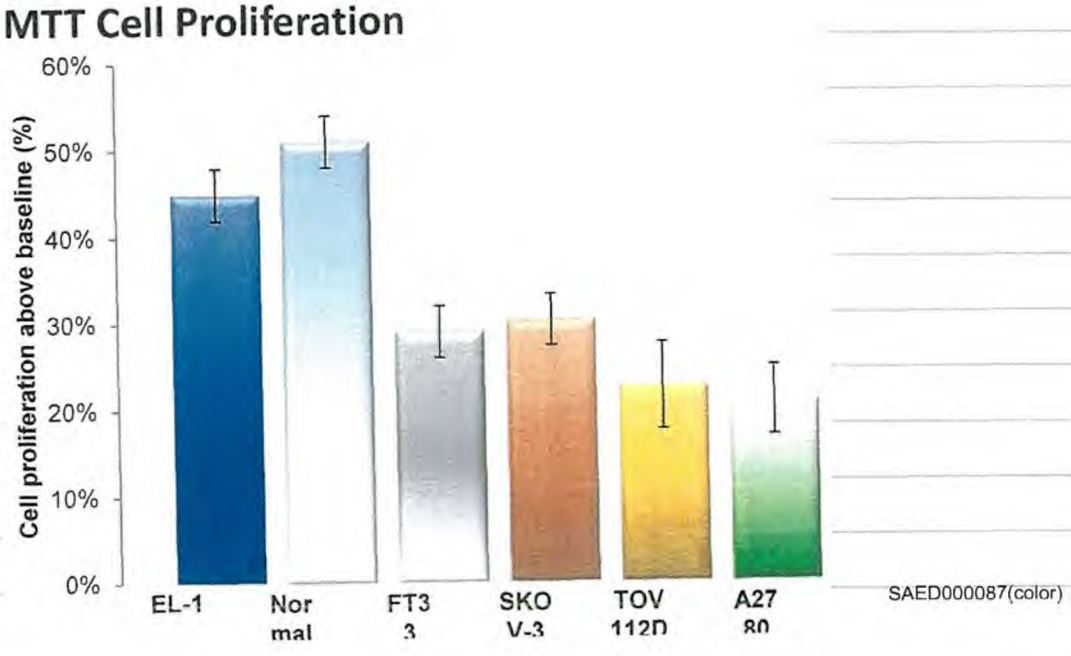
SAED000086(color)

106

Raw data

9/6/2018					
1	2	3	4	5	6
0.1764	0.17	0.1767	0.1616	0.15	0.156
0.212	0.223	0.2261	0.2899	0.2873	0.2719
0.1225	0.1248	0.1232	0.192	0.2087	0.1961
0.2198	0.2126	0.2171	0.2604	0.251	0.2598
0.3042	0.3017	0.3269	0.1383	0.1402	0.1437
0.1593	0.1506	0.1598	0.253	0.2643	0.2539
0.1244	0.1202	0.1282	0.151	0.1541	0.15
0.103	0.115	0.112	0.1411	0.1414	0.1408
0.225	0.2248	0.2232	0.192	0.2087	0.1961

Cell type	OD 1	OD 2	OD 3	Corr 1	Corr 2	Corr 3	Cytotoxicty (%) 1	Cytotoxicty (%) 2	Cytotoxicty (%) 3	Average	SD
A2780 unt	0.1764	0.17	0.1767	0.1764	0.17	0.1767	0%	0%	0%	0%	0%
100 ug/ml	0.212	0.223	0.2261	0.212	0.223	0.2261	17%	24%	22%	20.80%	4%
SKOV unt	0.2198	0.2126	0.2171	0.2198	0.2126	0.2171	0%	0%	0%	0.00%	0%
100 ug/ml	0.3042	0.3017	0.3269	0.3042	0.3017	0.3269	28%	30%	34%	30.29%	3%
TOV112 unt	0.192	0.2087	0.1961	0.192	0.2087	0.1961	0%	0%	0%	0.00%	0%
100 ug/ml	0.2604	0.251	0.2598	0.2604	0.251	0.2598	26%	17%	25%	22.55%	5%
EL-1 unt	0.1616	0.15	0.156	0.1616	0.15	0.156	0%	0%	0%	0.00%	0%
100 ug/ml	0.2899	0.2873	0.2719	0.2899	0.2873	0.2719	44%	48%	43%	44.89%	3%
Normal ovarian unt	0.103	0.115	0.112	0.103	0.115	0.112	0%	0%	0%	0.0%	0%
100 ug/ml	0.225	0.2248	0.2232	0.225	0.2248	0.2232	54%	49%	50%	51.0%	3%
FT33 unt	0.1411	0.1414	0.1408	0.1411	0.1414	0.1408	0%	0%	0%	0.0%	0%
100 ug/ml	0.192	0.2087	0.1961	0.192	0.2087	0.1961	27%	32%	28%	29.0%	3%

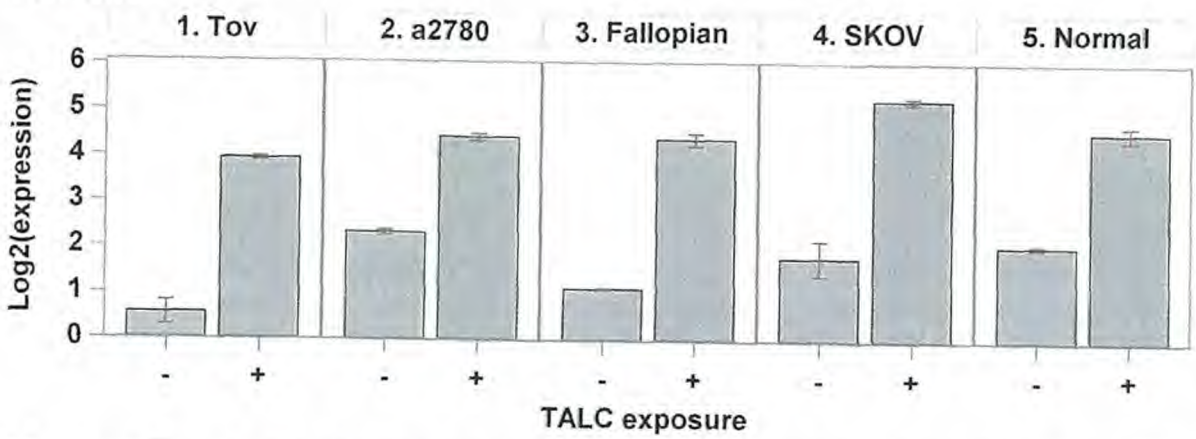


Statistical Sig Her Analysis

Statistical Analysis 10.6.18

- Normality was examined using the Kolmogorov-Simirnov test and by visual inspection of quantile-quantile plots.
- Because most of the data were not normally distributed, differences in distributions were examined using the Kruskal-Wallists test.
- Generalized linear models were used to examine pairwise differences in estimated least squares means by exposure to 0, 5, 20 or 100 ug/ml of Talc, with or without Tukey-Kramer adjustment for multiple comparisons.
- Analyte expression values were log2 transformed after adding a numeric constant "1" to avoid negative values.
- P-values below 0.05 was considered statistically significant

CA125



Mean +/- Standard Deviation Log2(Marker) Expression with and without exposure to TALC [Note: The data were log2 transformed after adding a numeric constant ('1') to avoid negative transformed expression values]

SAED000088(color)

Mean Std Med iQr PCR

PCR									
Analysis Variable : log2expPlusOne									
Cell_line	Marker	exposure	N Obs	Mean	Std Dev	Std Error	Median	25th Pctl	75th Pctl
A2780	CAT	i. 0 ug/ml	3	3.6569399	0.0366785	0.0500438	3.6474296	3.5916794	3.7617108
		ii. 5 ug/m	3	3.551069	0.0962913	0.0555938	3.5008021	3.4903134	3.6620915
		iii. 20 ug	3	3.0529977	0.0522557	0.0301698	3.0282155	3.0177438	3.1130337
		iv. 100 ug	3	2.3093358	0.0943387	0.0544665	2.2871768	2.228049	2.4127815
	GSR	i. 0 ug/ml	3	2.4390697	0.0218641	0.0126233	2.448108	2.4141355	2.4549655
		ii. 5 ug/m	3	2.3042019	0.0085862	0.0049573	2.2992447	2.2992447	2.3141164
		iii. 20 ug	3	1.8875253	0	0	1.8875253	1.8875253	1.8875253
		iv. 100 ug	3	1.4494482	0.0470991	0.0271927	1.4766409	1.3950628	1.4766409
	GST	i. 0 ug/ml	3	2.6387682	0.028132	0.016242	2.8225262	2.8225262	2.8712523
		ii. 5 ug/m	3	2.5891539	0.0820301	0.0473601	2.5604704	2.5253172	2.6316741
		iii. 20 ug	3	2.1458805	0.1374958	0.0793832	2.1528324	2.0050406	2.2797684
		iv. 100 ug	3	1.4536326	0.1735074	0.1001745	1.421156	1.2986583	1.6410836
	MPO	i. 0 ug/ml	3	4.4203917	0.3911882	0.2258526	4.4919172	3.9983761	4.7708819
		ii. 5 ug/m	3	5.4678059	0.0287839	0.0166184	5.4783894	5.4352285	5.4897998
		iii. 20 ug	3	6.1434159	0.0387315	0.0223617	6.1642432	6.0987269	6.1672776
		iv. 100 ug	3	6.5277399	0.0576635	0.0332921	6.5248159	6.4715939	6.5868098
	NO2	i. 0 ug/ml	3	4.0269499	0.0465503	0.0268758	4.0362396	3.9764553	4.0681549
		ii. 5 ug/m	3	4.3957668	0.0386617	0.0223213	4.3782342	4.3689778	4.4400884
		iii. 20 ug	3	4.7555891	0.0207424	0.0119757	4.7457751	4.7415748	4.7794173
		iv. 100 ug	3	5.4497071	0.3718927	0.2147123	5.2435548	5.2265471	5.8790195
	SOD	i. 0 ug/ml	3	4.7346013	0.0833955	0.0481484	4.7342219	4.6513962	4.8181859
		ii. 5 ug/m	3	4.3928697	0.1958249	0.1130596	4.3761514	4.2059398	4.5965178
		iii. 20 ug	3	3.362088	0.2905107	0.1677264	3.3589588	3.1038333	3.6834719
		iv. 100 ug	3	2.7808886	0.0229483	0.0132492	2.7924389	2.75446	2.7957669
EL-1	CAT	i. 0 ug/ml	3	4.6347343	0.007743	0.0044704	4.6320937	4.628657	4.6434522
		ii. 5 ug/m	3	4.4475437	0.0165619	0.0107283	4.4570689	4.4261305	4.4594316
		iii. 20 ug	3	3.9117219	0.0631814	0.0364778	3.891322	3.8612606	3.9825829
		iv. 100 ug	3	3.4137774	0.085582	0.0494108	3.3686287	3.3602238	3.5124797
	GSR	i. 0 ug/ml	3	3.1584852	0.0170451	0.009841	3.1529946	3.1448621	3.1775989
		ii. 5 ug/m	3	2.8776443	0.0207752	0.0119946	2.8710551	2.8609628	2.9009151
		iii. 20 ug	3	2.3091592	0.0085862	0.0049573	2.3141164	2.2992447	2.3141164
		iv. 100 ug	3	1.6341862	0.0494412	0.0285449	1.650305	1.5786972	1.6735564
	GST	i. 0 ug/ml	3	2.5764269	0.0731098	0.0422099	2.6095186	2.4928223	2.6271399
		ii. 5 ug/m	3	2.1904434	0.0169111	0.0097636	2.1906149	2.1734473	2.2072682
		iii. 20 ug	3	2.0394084	0.1873334	0.108157	2.1384868	1.8233418	2.1563966
		iv. 100 ug	3	1.2697114	0.1463694	0.0845064	1.2986583	1.1110313	1.3994446
	MPO	i. 0 ug/ml	3	6.3838911	0.190338	0.1098917	6.3861557	6.1924309	6.5730867
		ii. 5 ug/m	3	6.6957103	0.0395005	0.0228056	6.6743477	6.6714915	6.7412917
		iii. 20 ug	3	6.9337227	0.0236576	0.0136587	6.9360372	6.908993	6.956138
		iv. 100 ug	3	7.1102543	0.0261662	0.0151071	7.1212745	7.0803805	7.1291078
	NO2	i. 0 ug/ml	3	3.6938228	0.1276474	0.0736972	3.7208257	3.5548344	3.8058083
		ii. 5 ug/m	3	4.2260657	0.1170831	0.0675979	4.2039842	4.1215957	4.3526173
		iii. 20 ug	3	5.1669652	0.0562575	0.0324803	5.1839628	5.1041689	5.2127639
		iv. 100 ug	3	5.4177992	0.0803845	0.04641	5.41792	5.3373543	5.4981232
	SOD	i. 0 ug/ml	3	5.456788	0.3237203	0.1869	5.4515079	5.1357401	5.7831161
		ii. 5 ug/m	3	4.6439809	0.011235	0.0064865	4.6374943	4.6374943	4.6569539
		iii. 20 ug	3	3.9340229	0.0675847	0.03902	3.9214363	3.8736164	4.0070161
		iv. 100 ug	3	3.6529395	0.0577189	0.033324	3.6507646	3.5963389	3.7117151
FT33	CAT	i. 0 ug/ml	3	4.7632398	0.0189612	0.0109473	4.753658	4.7509816	4.7850797
		ii. 5 ug/m	3	4.5434772	0.0186948	0.0107935	4.5531147	4.5219302	4.5553867
		iii. 20 ug	3	3.9261712	0.0739331	0.0426853	3.9585643	3.8415706	3.9783787
		iv. 100 ug	3	3.3119478	0.0452928	0.0261498	3.3033424	3.2715751	3.3609261
	GSR	i. 0 ug/ml	3	2.6664232	0.0114767	0.0066261	2.6665294	2.6546938	2.6778465
		ii. 5 ug/m	3	2.2891742	0.0174426	0.0100705	2.2992447	2.2690331	2.2992447
		iii. 20 ug	3	1.8739905	0.0305612	0.0176445	1.8675012	1.8471952	1.9072753
		iv. 100 ug	3	1.0007073	0.0565114	0.0326268	1.0136409	0.9388503	1.0496308
	GST	i. 0 ug/ml	3	2.7078651	0.0109679	0.0063323	2.7125958	2.6953257	2.7156738
		ii. 5 ug/m	3	2.311642	0.0154105	0.0088972	2.3109217	2.2966042	2.3273999
		iii. 20 ug	3	1.9325492	0.0083186	0.0048027	1.9328171	1.9240999	1.9407308
		iv. 100 ug	3	1.0931968	0.1387384	0.0801006	1.1130337	0.9456077	1.220949
	MPO	i. 0 ug/ml	3	3.6837367	0.0521848	0.0301289	3.6595821	3.6460052	3.7436227
		ii. 5 ug/m	3	2.4202895	0.2581914	0.1490669	2.3527585	2.2025744	2.7055356
		iii. 20 ug	3	3.4310737	0.383192	0.221236	3.4238471	3.0515461	3.8178279
		iv. 100 ug	3	2.4868309	0.5736839	0.3312165	2.5043662	1.9045804	3.0515461
	NO2	i. 0 ug/ml	3	2.979957	0.1311209	0.0757027	3.0428194	2.8292407	3.0678108
		ii. 5 ug/m	3	4.2315124	0.1109416	0.0640522	4.211791	4.1317541	4.3509922
		iii. 20 ug	3	5.0423074	0.0505578	0.0291896	5.0403226	4.9943082	5.0952915
		iv. 100 ug	3	5.2273225	0.0176408	0.0101849	5.2222636	5.2127639	5.2469401
	SOD	i. 0 ug/ml	3	4.2502737	0.1042203	0.0601716	4.2540849	4.1442096	4.3525467
		ii. 5 ug/m	3	3.5078828	0.0672157	0.038807	3.4736571	3.4646683	3.5853231
		iii. 20 ug	3	2.5079502	0.0562015	0.032448	2.4985062	2.4570689	2.5682754

RCR

		iv. 100 ug	3	1.9444382	0.0880196	0.0508182	1.937721	1.8599695	2.0355239
NOE	CAT	i. 0 ug/ml	3	3.710353	0.048923	0.0282457	3.7026575	3.6657338	3.7626677
		ii. 5 ug/m	3	3.4435567	0.0146987	0.0084863	3.4408193	3.4304191	3.4594316
		iii. 20 ug	3	3.0660752	0.0478082	0.0276021	3.0499792	3.0283923	3.1198541
		iv. 100 ug	3	2.4488001	0.0523535	0.0302263	2.4709273	2.3890161	2.486457
	GSR	i. 0 ug/ml	3	3.2666551	0.0315951	0.0182414	3.2567088	3.24123	3.3020265
		ii. 5 ug/m	3	2.9555529	0.0239087	0.0138037	2.9588427	2.9301696	2.9776483
		iii. 20 ug	3	2.6352146	0.0180554	0.0104243	2.6313371	2.619413	2.6548936
		iv. 100 ug	3	2.2382223	0.0154412	0.008915	2.2381754	2.2228046	2.2536868
	GST	i. 0 ug/ml	3	2.4084654	0.3030423	0.1749615	2.5675454	2.0590091	2.5988416
		ii. 5 ug/m	3	1.9560063	0.0358575	0.0207023	1.9437338	1.9278965	1.9963887
		iii. 20 ug	3	1.4784848	0.0929867	0.0536859	1.5265697	1.3712801	1.5375446
		iv. 100 ug	3	0.7539717	0.0296043	0.0170921	0.7432995	0.7311832	0.7874325
	MPO	i. 0 ug/ml	3	3.3801234	0.0349896	0.0202012	3.3804524	3.3449705	3.4149473
		ii. 5 ug/m	3	2.709032	0.4784764	0.2762485	2.7710404	2.2025744	3.1534811
		iii. 20 ug	3	2.9606787	0.0339471	0.0195994	2.9703015	2.922959	2.9887755
		iv. 100 ug	3	2.8974492	0.2328767	0.1344514	2.8641356	2.6830227	3.1451883
	NO2	i. 0 ug/ml	3	3.7243216	0.0501246	0.0289395	3.7086287	3.683921	3.7804151
		ii. 5 ug/m	3	4.394039	0.0368408	0.0212701	4.375943	4.3697456	4.4364284
		iii. 20 ug	3	4.928477	0.0549816	0.0317436	4.9279438	4.873764	4.9837233
		iv. 100 ug	3	5.5337093	0.0347537	0.0200651	5.5276334	5.5023943	5.5711003
	SOD	i. 0 ug/ml	3	3.9632817	0.1567405	0.0904942	3.9799339	3.79888	4.1110313
		ii. 5 ug/m	3	3.5676281	0.0822589	0.0474922	3.5279461	3.5127326	3.6622055
		iii. 20 ug	3	2.6525607	0.0620037	0.0357978	2.6782973	2.5818333	2.6975514
		iv. 100 ug	3	2.3672706	0.0899191	0.0519148	2.3631711	2.2794713	2.4591693
SKOV-3	CAT	i. 0 ug/ml	3	3.8929757	0.1268283	0.0732244	3.9657843	3.7465278	3.966615
		ii. 5 ug/m	3	3.5294147	0.108096	0.0624093	3.4767705	3.4577256	3.6537479
		iii. 20 ug	3	3.2783228	0.1368648	0.079019	3.2509618	3.1572054	3.4268015
		iv. 100 ug	3	2.2973771	0.0509856	0.0294365	2.3193289	2.2390925	2.33571
	GSR	i. 0 ug/ml	3	3.0022571	0.0104774	0.0060491	2.9962079	2.9962079	3.0143553
		ii. 5 ug/m	3	2.4277015	0.0273508	0.015791	2.4278743	2.4002647	2.4549655
		iii. 20 ug	3	2.2841653	0.0151058	0.0087214	2.284218	2.2690331	2.2992447
		iv. 100 ug	3	1.7840822	0.0427295	0.0246699	1.784504	1.7411434	1.8265993
	GST	i. 0 ug/ml	3	2.7222988	0.0512982	0.0296171	2.7422218	2.6640277	2.7606468
		ii. 5 ug/m	3	2.4610932	0.0675923	0.0390245	2.4926223	2.3834969	2.5071603
		iii. 20 ug	3	2.1321947	0.11075	0.0639415	2.1839528	2.0050406	2.2075805
		iv. 100 ug	3	1.5233864	0.0812994	0.0469382	1.5533605	1.4313555	1.5854433
	MPO	i. 0 ug/ml	3	4.3390025	0.0414058	0.0239057	4.3382105	4.2979984	4.3807987
		ii. 5 ug/m	3	5.796017	0.0163462	0.0094375	5.8048022	5.7771567	5.806092
		iii. 20 ug	3	6.362497	0.0311108	0.0179619	6.3553159	6.3356047	6.3965705
		iv. 100 ug	3	6.9662174	0.0006784	0.0003917	6.9678257	6.9678257	6.9690008
	NO2	i. 0 ug/ml	3	4.0903869	0.0303827	0.0175415	4.1030779	4.0557163	4.1123665
		ii. 5 ug/m	3	4.3082108	0.0500746	0.0289106	4.3192566	4.2535355	4.3518403
		iii. 20 ug	3	4.7663444	0.0337166	0.0194663	4.760006	4.7395781	4.8054472
		iv. 100 ug	3	5.2241662	0.0317886	0.0183531	5.2131917	5.199319	5.259988
	SOD	i. 0 ug/ml	3	4.6762514	0.1489147	0.0859759	4.6402739	4.5486216	4.8398588
		ii. 5 ug/m	3	4.119983	0.0126658	0.0073126	4.1171963	4.1089426	4.1338101
		iii. 20 ug	3	3.4742309	0.1798399	0.1038306	3.4603494	3.301734	3.6606092
		iv. 100 ug	3	2.8485275	0.0896049	0.0518489	2.8341044	2.7688072	2.9446711
TOV-112	CAT	i. 0 ug/ml	3	3.9367605	0.0147273	0.0085028	3.9307373	3.9259994	3.9535446
		ii. 5 ug/m	3	3.6047714	0.0070956	0.0040967	3.602053	3.5994368	3.6126243
		iii. 20 ug	3	2.9234085	0.0367419	0.021213	2.9305481	2.8836208	2.9560567
		iv. 100 ug	3	2.3772379	0.1346644	0.0777486	2.4138648	2.228049	2.4897998
	GSR	i. 0 ug/ml	3	2.3574049	0.0430757	0.0248698	2.3576335	2.3141164	2.4002847
		ii. 5 ug/m	3	2.1636806	0.011926	0.0068855	2.1705661	2.1499097	2.1705661
		iii. 20 ug	3	1.6028306	0.0239891	0.0138501	1.6031219	1.5786972	1.6266728
		iv. 100 ug	3	1.3281956	0.0169756	0.0098009	1.3379965	1.3085939	1.3379965
	GST	i. 0 ug/ml	3	2.6358633	0.1267042	0.0731527	2.6594679	2.4990167	2.7491051
		ii. 5 ug/m	3	2.2357771	0.0874612	0.0504957	2.1940871	2.176961	2.3362834
		iii. 20 ug	3	1.5685321	0.1467886	0.0847485	1.5509007	1.4313555	1.7238401
		iv. 100 ug	3	0.9064236	0.1433487	0.0827524	0.8519988	0.7982579	1.0690147
	MPO	i. 0 ug/ml	3	3.8316651	0.1626979	0.0939337	3.8546319	3.6550083	3.975355
		ii. 5 ug/m	3	4.7174873	0.1407705	0.0812739	4.7681843	4.5583901	4.8258874
		iii. 20 ug	3	5.2355859	0.0746043	0.0430728	5.2271635	5.1655502	5.3140439
		iv. 100 ug	3	6.4598527	0.02951	0.0170376	6.4509143	6.4358452	6.4927986
	NO2	i. 0 ug/ml	3	3.8861832	0.10971	0.0633411	3.8598702	3.7920224	4.0066571
		ii. 5 ug/m	3	4.5915294	0.1000141	0.0577432	4.5760383	4.5001647	4.6983852
		iii. 20 ug	3	4.3025066	0.0619684	0.0357775	4.3112125	4.2366457	4.3596617
		iv. 100 ug	3	5.1023523	0.1002657	0.0578885	5.1231695	4.9933121	5.1905754
	SOD	i. 0 ug/ml	3	5.0877115	0.0207012	0.0119519	5.0557596	5.0557596	5.0916152
		ii. 5 ug/m	3	4.34704	0.1709547	0.0987007	4.3431235	4.1780772	4.5199193
		iii. 20 ug	3	3.360278	0.1217864	0.0703019	3.33371	3.2539893	3.4831349
		iv. 100 ug	3	2.8462591	0.0881635	0.0509012	2.8308638	2.7668072	2.9411063

Mean Std Med iQr ELISA

ELISA

Analysis Variable : log2expPlusOne

Cell_Line	Marker	exposure	N Obs	Mean	Std Dev	Std Error	Median	25th Pctl	75th Pctl
A2780	CAT	i. 0 ug/ml	3	4.2574633	3.6874777	2.1289663	6.3314549	0	6.4409351
		ii. 5 ug/m	3	5.9586593	0.1269159	0.0732749	6.0147182	5.8133665	6.0478932
		iii. 20 ug	3	5.4206681	0.1545027	0.0892022	5.4420085	5.2566045	5.5633913
		iv. 100 ug	3	3.3871439	0.1580791	0.091267	3.3030442	3.2888925	3.5894949
	GSR	i. 0 ug/ml	3	2.9264839	0.0560937	0.0323857	2.9565787	2.8617654	2.9611097
		ii. 5 ug/m	3	1.8449008	0.5332502	0.3078721	2.1104274	1.2310183	2.1932582
		iii. 20 ug	3	2.6310649	0.1962989	0.1133332	2.5569484	2.4748412	2.8514049
		iv. 100 ug	3	1.9299141	0.1596065	0.0921488	1.9115039	1.780311	2.0979273
	GSTp1	i. 0 ug/ml	3	5.7815173	0.592863	0.3422896	5.6515447	5.2644238	6.4285833
		ii. 5 ug/m	3	5.2209741	0.0698484	0.040327	5.2357195	5.1449303	5.2822726
		iii. 20 ug	3	4.4893251	0.200935	0.1160099	4.5497155	4.2851206	4.6531392
		iv. 100 ug	3	2.864807	0.1177024	0.0679555	2.9145098	2.7304062	2.9495049
	MPO	i. 0 ug/ml	3	0.1695431	0.0354213	0.0204505	0.1615308	0.1388142	0.2082842
		ii. 5 ug/m	3	0.3337243	0.043782	0.0252776	0.3514984	0.2838504	0.3858241
		iii. 20 ug	3	0.4814097	0.0205819	0.0118829	0.4910932	0.4577721	0.4953639
		iv. 100 ug	3	0.9288378	0.0655643	0.0378536	0.9442591	0.8569375	0.985317
	SOD3	i. 0 ug/ml	3	1.6356913	0.0687084	0.0396688	1.6205848	1.5757932	1.7106959
		ii. 5 ug/m	3	1.3851168	0.0391983	0.0226312	1.3644345	1.3605913	1.4303248
		iii. 20 ug	3	1.0724011	0.0370578	0.0213953	1.087577	1.0301642	1.099462
		iv. 100 ug	3	0.571832	0.0450738	0.0260234	0.5822635	0.522457	0.6107754
	iNOS	i. 0 ug/ml	3	2.6881765	0.0984259	0.0568262	2.6911032	2.5883199	2.7851065
		ii. 5 ug/m	3	3.2130977	0.0487256	0.0281317	3.1855487	3.1843872	3.2693572
		iii. 20 ug	3	4.1128539	0.0650274	0.0375436	4.0981837	4.0564148	4.1839633
		iv. 100 ug	3	4.5493201	0.0334892	0.019335	4.5523704	4.51441	4.5811798
EL1	CAT	i. 0 ug/ml	3	6.3300792	1.0158524	0.5865026	6.8414955	5.1601522	6.9885899
		ii. 5 ug/m	3	6.4719538	0.2664051	0.153809	6.4819999	6.2006678	6.7331937
		iii. 20 ug	3	5.8725758	0.0690101	0.039843	5.8582776	5.8131736	5.9482761
		iv. 100 ug	3	5.2392865	0.172504	0.0995952	5.2924379	5.0464816	5.3789602
	GSR	i. 0 ug/ml	3	5.1198603	0.2068003	0.1193962	5.0403899	4.9645866	5.3546084
		ii. 5 ug/m	3	4.1921745	0.5224649	0.3016452	4.4293835	3.5931864	4.5539537
		iii. 20 ug	3	2.2632358	0.0992817	0.0573203	2.2588513	2.166219	2.3646371
		iv. 100 ug	3	2.6260068	0.112917	0.0651927	2.6324997	2.5099835	2.7355373
	GSTp1	i. 0 ug/ml	3	5.3269582	0.0255202	0.0147341	5.3210982	5.3048777	5.3548968
		ii. 5 ug/m	3	4.3978426	0.3052705	0.176248	4.4171413	4.0833807	4.6930059
		iii. 20 ug	3	4.7882778	0.0915836	0.0528758	4.8217331	4.6846702	4.8584299
		iv. 100 ug	3	4.6385363	0.3957429	0.2284823	4.47636	4.3496426	5.0896064
	MPO	i. 0 ug/ml	3	0.590605	0.0020024	0.0011561	0.5916646	0.5882954	0.591855
		ii. 5 ug/m	3	0.4965263	0.1202534	0.0694283	0.5295436	0.3632133	0.5968221
		iii. 20 ug	3	0.7353674	0.034122	0.0197003	0.7317707	0.7031862	0.7711452
		iv. 100 ug	3	2.6882919	0.1356558	0.0783209	2.7400205	2.5343623	2.790473
	SOD3	i. 0 ug/ml	3	1.9403529	0.0038349	0.0022141	1.9423824	1.9359298	1.9427466
		ii. 5 ug/m	3	1.782667	0.0262256	0.0151413	1.7894473	1.753717	1.8048365
		iii. 20 ug	3	1.402393	0.0115761	0.0066835	1.4011151	1.3915089	1.4145551
		iv. 100 ug	3	1.0514554	0.0420738	0.0242913	1.0329162	1.0218349	1.099615
	iNOS	i. 0 ug/ml	3	1.1485613	0.0486258	0.0280741	1.1221945	1.1188138	1.2046757
		ii. 5 ug/m	3	1.8412055	0.0516723	0.029833	1.822735	1.8013068	1.8995748
		iii. 20 ug	3	3.0092736	0.0421275	0.0243223	3.0097882	2.9668911	3.0511415
		iv. 100 ug	3	4.5040677	0.0646358	0.0373175	4.5045345	4.4391998	4.5684688
FT33	CAT	i. 0 ug/ml	3	5.9332377	0.1536457	0.0887074	5.9078581	5.793862	6.097993
		ii. 5 ug/m	3	5.5351585	0.030892	0.0178355	5.523883	5.5114881	5.5701043
		iii. 20 ug	3	4.9708572	0.0418055	0.0241364	4.9772722	4.926215	5.0090845
		iv. 100 ug	3	2.4514373	0.1291939	0.0745902	2.5223921	2.3023151	2.5296046
	GSR	i. 0 ug/ml	3	3.5339547	0.0596797	0.0345715	3.504263	3.4947421	3.6028889
		ii. 5 ug/m	3	2.8607994	0.0235767	0.013612	2.8650113	2.8354007	2.8819864
		iii. 20 ug	3	2.0278518	0.0290597	0.0167776	2.0378526	1.9951124	2.0505904
		iv. 100 ug	3	1.8968547	0.0231513	0.0133684	1.8984466	1.8729485	1.919169
	GSTp1	i. 0 ug/ml	3	4.7361471	0.107903	0.0622978	4.7079212	4.6451624	4.8553578
		ii. 5 ug/m	3	4.327875	0.1985287	0.1146208	4.4131073	4.1009622	4.4695555
		iii. 20 ug	3	4.1330676	0.0801956	0.0463009	4.1048186	4.0708207	4.2235655
		iv. 100 ug	3	3.7795494	0.0577846	0.0333619	3.7815482	3.7207913	3.8363086
	MPO	i. 0 ug/ml	3	0.1080559	0.0087403	0.0050462	0.1056055	0.1008025	0.1177599
		ii. 5 ug/m	3	0.1422642	0.0022615	0.0013057	0.1421418	0.1400664	0.1445843
		iii. 20 ug	3	0.0365338	0.0050604	0.0029216	0.0377899	0.0309636	0.0408479
		iv. 100 ug	3	0.0562339	0.0028877	0.0016672	0.0576544	0.052911	0.0581361
	SOD3	i. 0 ug/ml	3	1.5787641	0.0592452	0.0342053	1.6055748	1.5108529	1.6198646
		ii. 5 ug/m	3	1.2077143	0.0591121	0.0341284	1.2189842	1.1437785	1.2603801
		iii. 20 ug	3	1.1026223	0.0814474	0.0470237	1.1148175	1.015765	1.1772845
		iv. 100 ug	3	0.2736437	0.0096006	0.0055429	0.2790651	0.2625588	0.2793072
	iNOS	i. 0 ug/ml	3	2.1834173	0.0165584	0.00956	2.184654	2.1682753	2.1993227
		ii. 5 ug/m	3	3.0461442	0.0574143	0.0331482	3.0523119	2.9858951	3.1002257
		iii. 20 ug	3	3.8189895	0.0362527	0.0220852	3.807153	3.7880542	3.8617614

ELISA

		iv. 100 ug	3	4.3384287	0.0174581	0.0100794	4.3344887	4.3232772	4.3575201
NOE	CAT	i. 0 ug/ml	3	6.7716206	0.013433	0.0077555	6.7743003	6.7570498	6.7835118
		ii. 5 ug/m	3	5.1019337	0.1200419	0.0693062	5.0744766	4.9979991	5.2333255
		iii. 20 ug	3	4.2849863	0.197989	0.114309	4.3209484	4.0714811	4.4625294
		iv. 100 ug	3	2.4565889	0.0742726	0.0428813	2.438142	2.3932782	2.5383465
	GSR	i. 0 ug/ml	3	3.494584	0.0675773	0.0390158	3.5123609	3.4198952	3.5514958
		ii. 5 ug/m	3	3.2496606	0.2901599	0.1675239	3.3439705	2.924078	3.4809333
		iii. 20 ug	3	2.4497142	0.1566082	0.0904178	2.4748103	2.2820734	2.592259
		iv. 100 ug	3	2.3945716	0.0941968	0.0543846	2.4130934	2.2924896	2.4781317
	GSTp1	i. 0 ug/ml	3	6.3242069	0.0418843	0.0241819	6.3136459	6.2886139	6.3703609
		ii. 5 ug/m	3	5.8542165	0.0546727	0.0315653	5.8698997	5.7934162	5.8993337
		iii. 20 ug	3	4.9553189	0.1116465	0.0644591	4.9305681	4.8581248	5.0772639
		iv. 100 ug	3	3.5904539	0.0051691	0.0029844	3.591323	3.5849054	3.5951334
	MPO	i. 0 ug/ml	3	0.1295906	0.0072796	0.0042029	0.128816	0.1227292	0.1372265
		ii. 5 ug/m	3	0.0362084	0.0013902	0.00080262	0.0364896	0.0348991	0.0374365
		iii. 20 ug	3	0.0595687	0.0059828	0.0034542	0.0565222	0.0557223	0.0664616
		iv. 100 ug	3	0.0897481	0.0032252	0.0018621	0.0898807	0.0864586	0.0929051
	SOD3	i. 0 ug/ml	3	1.6738595	0.0447064	0.0258113	1.6922109	1.6229094	1.7064883
		ii. 5 ug/m	3	1.47609	0.0516773	0.0298359	1.4920505	1.4183153	1.5179043
		iii. 20 ug	3	1.1836494	0.0408426	0.0235805	1.20437	1.1365998	1.2099785
		iv. 100 ug	3	0.6498753	0.0215272	0.0124287	0.6443161	0.6316729	0.6736367
	iNOS	i. 0 ug/ml	3	2.8443782	0.043948	0.0253734	2.8627272	2.7942292	2.8761783
		ii. 5 ug/m	3	3.5390046	0.0471559	0.0272254	3.5516972	3.4868014	3.578515
		iii. 20 ug	3	4.0982555	0.0313536	0.018102	4.0809164	4.0794012	4.1344489
		iv. 100 ug	3	4.7720961	0.0576565	0.033288	4.791697	4.7071946	4.8173966
SKOV3	CAT	i. 0 ug/ml	3	6.2349114	0.0800568	0.0462208	6.1902817	6.1871175	6.327335
		ii. 5 ug/m	3	5.1019337	0.1200419	0.0693062	5.0744766	4.9979991	5.2333255
		iii. 20 ug	3	4.2849863	0.197989	0.114309	4.3209484	4.0714811	4.4625294
		iv. 100 ug	3	2.299037	1.0279868	0.5935084	2.8653202	1.1124302	2.9193605
	GSR	i. 0 ug/ml	3	3.494584	0.0675773	0.0390158	3.5123609	3.4198952	3.5514958
		ii. 5 ug/m	3	3.2496606	0.2901599	0.1675239	3.3439705	2.924078	3.4809333
		iii. 20 ug	3	2.4497142	0.1566082	0.0904178	2.4748103	2.2820734	2.592259
		iv. 100 ug	3	2.3945716	0.0941968	0.0543846	2.4130934	2.2924896	2.4781317
	GSTp1	i. 0 ug/ml	3	6.9973966	0.0983728	0.0567955	6.9492458	6.9323747	7.1105692
		ii. 5 ug/m	3	6.3242069	0.0418843	0.0241819	6.3136459	6.2886139	6.3703609
		iii. 20 ug	3	5.4134255	0.074402	0.042956	5.4350046	5.3306181	5.4746527
		iv. 100 ug	3	5.0327546	0.0887407	0.0512345	5.0772639	4.9305681	5.0904318
	MPO	i. 0 ug/ml	3	0.0691673	0.0126831	0.0073226	0.0632442	0.0605294	0.0837264
		ii. 5 ug/m	3	0.1206415	0.0148581	0.0085783	0.128816	0.103491	0.1296173
		iii. 20 ug	3	0.304674	0.0379414	0.0219055	0.3196048	0.2615385	0.3328788
		iv. 100 ug	3	0.8643402	0.18529	0.1069772	0.9375621	0.6536279	1.0018307
	SOD3	i. 0 ug/ml	3	1.6647907	0.0693283	0.0400267	1.6266476	1.6229094	1.744815
		ii. 5 ug/m	3	1.4793007	0.0463265	0.0267466	1.4857385	1.430092	1.5220717
		iii. 20 ug	3	1.1773978	0.1118808	0.0645944	1.2283631	1.0491085	1.2547219
		iv. 100 ug	3	0.5292229	0.1245649	0.0719176	0.5776365	0.3877194	0.6223127
	iNOS	i. 0 ug/ml	3	2.9574573	0.0279231	0.0161214	2.9656585	2.926352	2.9803614
		ii. 5 ug/m	3	3.7412353	0.0213938	0.0123517	3.749426	3.7169563	3.7573236
		iii. 20 ug	3	4.3598372	0.0494917	0.028574	4.3573902	4.3116144	4.410507
		iv. 100 ug	3	4.7566821	0.1622307	0.093664	4.6696177	4.6565702	4.9438585
TOV112	CAT	i. 0 ug/ml	3	5.9325442	0.2328765	0.1344513	5.982594	5.6787121	6.1363265
		ii. 5 ug/m	3	5.5465926	0.0223311	0.0128929	5.5472347	5.5239473	5.5685957
		iii. 20 ug	3	4.9538929	0.0387127	0.0223508	4.9463705	4.9194934	4.9958148
		iv. 100 ug	3	2.1420073	0.6212806	0.3586965	2.1110408	1.5367891	2.7781921
	GSR	i. 0 ug/ml	3	3.5339647	0.059797	0.0345715	3.504263	3.4947421	3.6028889
		ii. 5 ug/m	3	2.8607994	0.0235767	0.013612	2.8650113	2.8354007	2.8819964
		iii. 20 ug	3	2.0278518	0.0290597	0.0167776	2.0378526	1.9951124	2.0505904
		iv. 100 ug	3	1.8968547	0.0231513	0.0133664	1.8984466	1.8729465	1.919169
	GSTp1	i. 0 ug/ml	3	5.2904247	0.2022888	0.1167915	5.3850267	5.0581679	5.4280794
		ii. 5 ug/m	3	5.0424775	0.0018888	0.0010905	5.0427937	5.0404505	5.0441882
		iii. 20 ug	3	4.7361471	0.107903	0.0622978	4.7079212	4.6451624	4.8553578
		iv. 100 ug	3	3.6942247	0.3135182	0.1810098	3.8317863	3.3354416	3.915446
	MPO	i. 0 ug/ml	3	0.2017849	0.0284656	0.0164346	0.1910605	0.1802392	0.2340549
		ii. 5 ug/m	3	0.4599654	0.0232861	0.0134442	0.4592672	0.4370363	0.4835927
		iii. 20 ug	3	0.8324438	0.050639	0.0292364	0.8104804	0.7964946	0.8903565
		iv. 100 ug	3	1.0260856	0.0144816	0.008361	1.0231644	1.0132873	1.0418052
	SOD3	i. 0 ug/ml	3	1.5117596	0.1226454	0.0708093	1.4920118	1.4001863	1.6430806
		ii. 5 ug/m	3	1.2403551	0.0442127	0.0255262	1.2624133	1.1894533	1.2691989
		iii. 20 ug	3	1.1290284	0.0969399	0.0559683	1.1305428	1.0313372	1.2251992
		iv. 100 ug	3	0.2597342	0.0132534	0.0076519	0.2570575	0.2480234	0.2741217
	iNOS	i. 0 ug/ml	3	2.1677451	0.0419739	0.0242337	2.1804552	2.1208851	2.2018949
		ii. 5 ug/m	3	3.0249777	0.1401724	0.0809286	3.0523119	2.8731515	3.1454697
		iii. 20 ug	3	3.7687098	0.049076	0.028334	3.7439537	3.7369426	3.8252331
		iv. 100 ug	3	4.2847345	0.0758406	0.0437866	4.2626797	4.2223657	4.3691579

- Non-parametric Kruskal-Wallis test for differences in distributions of each marker by exposure group;
- $P<0.05$ indicates to reject the null hypothesis that there is no difference in expression among the four exposure groups.

PCR				ELISA			
Cell_Line	Marker	Kruskal-Wallis	Nominal P-value, Kruskal-Wallis Test	Cell_Line	Marker	Kruskal-Wallis	Nominal P-value, Kruskal-Wallis Test
A2780	CAT	4.85	0.18	A2780	CAT	9.67	**0.02**
	GSR	9.46	**0.02**		GSR	10.61	**0.01**
	GSTp1	9.97	**0.02**		GST	10.42	**0.02**
	MPO	10.38	**0.02**		MPO	10.38	**0.02**
	SOD3	10.38	**0.02**		NO2	10.38	**0.02**
	iNOS	10.38	**0.02**		SOD	10.38	**0.02**
EL1	CAT	5.67	0.13	EL-1	CAT	10.38	**0.02**
	GSR	10.38	**0.02**		GSR	10.42	**0.02**
	GSTp1	7.51	0.06		GST	10.38	**0.02**
	MPO	9.46	**0.02**		MPO	10.38	**0.02**
	SOD3	10.38	**0.02**		NO2	10.38	**0.02**
	iNOS	10.38	**0.02**		SOD	10.42	**0.02**
FT33	CAT	10.38	**0.02**	FT33	CAT	10.38	**0.02**
	GSR	10.38	**0.02**		GSR	10.42	**0.02**
	GSTp1	9.67	**0.02**		GST	10.38	**0.02**
	MPO	10.38	**0.02**		MPO	8.07	**0.04**
	SOD3	9.97	**0.02**		NO2	10.38	**0.02**
	iNOS	10.38	**0.02**		SOD	10.38	**0.02**
NOE	CAT	10.38	**0.02**	NOE	CAT	10.38	**0.02**
	GSR	8.95	**0.03**		GSR	10.38	**0.02**
	GSTp1	10.38	**0.02**		GST	10.38	**0.02**
	MPO	10.38	**0.02**		MPO	6.59	0.09
	SOD3	10.38	**0.02**		NO2	10.38	**0.02**
	iNOS	10.38	**0.02**		SOD	10.38	**0.02**
SKOV3	CAT	10.38	**0.02**	SKOV-3	CAT	10.38	**0.02**
	GSR	8.95	**0.03**		GSR	10.42	**0.02**
	GSTp1	10.38	**0.02**		GST	10.38	**0.02**
	MPO	10.38	**0.02**		MPO	10.42	**0.02**
	SOD3	10.38	**0.02**		NO2	10.38	**0.02**
	iNOS	10.38	**0.02**		SOD	10.38	**0.02**
TOV112	CAT	10.38	**0.02**	TOV-112	CAT	10.38	**0.02**
	GSR	10.38	**0.02**		GSR	10.46	**0.02**
	GSTp1	10.38	**0.02**		GST	10.38	**0.02**
	MPO	10.38	**0.02**		MPO	10.38	**0.02**
	SOD3	9.97	**0.02**		NO2	10.38	**0.02**
	iNOS	10.38	**0.02**		SOD	10.42	**0.02**

Note: The data examined were log2 transformed after adding a numeric constant ('1') to avoid negative transformed expression values; the Kruskal-Wallis test had 3 degrees of freedom.

SAED000093(color)

General linear model results PCR

PCR

Differences by exposure :

Cell_Line	Marker	i/j	Nominal p-values: i. 0 ug/ml	ii. 5 ug/m	iii. 20 ug	iv. 100 ug	Tukey-Kramer Adjusted: i/j	i. 0 ug/ml	ii. 5 ug/m	iii. 20 ug	iv. 100 ug
A2780	CAT	i. 0 ug/ml	_	0.2923	0.463	0.58	i. 0 ug/ml	_	0.6843	0.8654	0.9363
A2780	CAT	ii. 5 ug/m	0.2923	_	0.7307	0.1268	ii. 5 ug/m	0.6843	_	0.9834	0.3812
A2780	CAT	iii. 20 ug	0.463	0.7307	_	0.2147	iii. 20 ug	0.8654	0.9834	_	0.5614
A2780	CAT	iv. 100 ug	0.58	0.1268	0.2147	_	iv. 100 ug	0.9363	0.3812	0.5614	_
A2780	GSR	i. 0 ug/ml	_	0.0021	0.257	0.0034	i. 0 ug/ml	_	0.009	0.6321	0.0142
A2780	GSR	ii. 5 ug/m	0.0021	_	0.0117	0.7345	ii. 5 ug/m	0.009	_	0.0469	0.9841
A2780	GSR	iii. 20 ug	0.257	0.0117	_	0.02	iii. 20 ug	0.6321	0.0469	_	0.077
A2780	GSR	iv. 100 ug	0.0034	0.7345	0.02	_	iv. 100 ug	0.0142	0.9841	0.077	_
A2780	GSTp1	i. 0 ug/ml	_	0.0645	0.0011	<.0001	i. 0 ug/ml	_	0.2189	0.005	<.0001
A2780	GSTp1	ii. 5 ug/m	0.0645	_	0.0233	<.0001	ii. 5 ug/m	0.2189	_	0.0886	<.0001
A2780	GSTp1	iii. 20 ug	0.0011	0.0233	_	0.0003	iii. 20 ug	0.005	0.0886	_	0.0012
A2780	GSTp1	iv. 100 ug	<.0001	<.0001	0.0003	_	iv. 100 ug	<.0001	<.0001	0.0012	_
A2780	MPO	i. 0 ug/ml	_	0.0019	<.0001	<.0001	i. 0 ug/ml	_	0.0084	0.0001	<.0001
A2780	MPO	ii. 5 ug/m	0.0019	_	0.0036	<.0001	ii. 5 ug/m	0.0084	_	0.0151	<.0001
A2780	MPO	iii. 20 ug	<.0001	0.0036	_	<.0001	iii. 20 ug	0.0001	0.0151	_	<.0001
A2780	MPO	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
A2780	SOD3	i. 0 ug/ml	_	0.0002	<.0001	<.0001	i. 0 ug/ml	_	0.0011	<.0001	<.0001
A2780	SOD3	ii. 5 ug/m	0.0002	_	<.0001	<.0001	ii. 5 ug/m	0.0011	_	0.0002	<.0001
A2780	SOD3	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	0.0002	_	<.0001
A2780	SOD3	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
A2780	iNOS	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001
A2780	iNOS	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001
A2780	iNOS	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	0.0002
A2780	iNOS	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	0.0002	_
EL1	CAT	i. 0 ug/ml	_	0.7529	0.3241	0.0366	i. 0 ug/ml	_	0.9872	0.7265	0.1334
EL1	CAT	ii. 5 ug/m	0.7529	_	0.2059	0.0221	ii. 5 ug/m	0.9872	_	0.5456	0.0844
EL1	CAT	iii. 20 ug	0.3241	0.2059	_	0.1839	iii. 20 ug	0.7265	0.5456	_	0.5038
EL1	CAT	iv. 100 ug	0.0366	0.0221	0.1839	_	iv. 100 ug	0.1334	0.0844	0.5038	_
EL1	GSR	i. 0 ug/ml	_	0.0045	<.0001	<.0001	i. 0 ug/ml	_	0.0189	<.0001	<.0001
EL1	GSR	ii. 5 ug/m	0.0045	_	<.0001	0.0002	ii. 5 ug/m	0.0189	_	0.0002	0.0008
EL1	GSR	iii. 20 ug	<.0001	<.0001	_	0.1651	iii. 20 ug	<.0001	0.0002	_	0.4659
EL1	GSR	iv. 100 ug	<.0001	0.0002	0.1651	_	iv. 100 ug	<.0001	0.0008	0.4659	_
EL1	GSTp1	i. 0 ug/ml	_	0.0021	0.0319	0.0106	i. 0 ug/ml	_	0.0089	0.118	0.0427
EL1	GSTp1	ii. 5 ug/m	0.0021	_	0.0969	0.2799	ii. 5 ug/m	0.0089	_	0.3077	0.6667
EL1	GSTp1	iii. 20 ug	0.0319	0.0969	_	0.4915	iii. 20 ug	0.118	0.3077	_	0.8861
EL1	GSTp1	iv. 100 ug	0.0106	0.2799	0.4915	_	iv. 100 ug	0.0427	0.6667	0.8861	_
EL1	MPO	i. 0 ug/ml	_	0.2469	0.0908	<.0001	i. 0 ug/ml	_	0.6161	0.2917	<.0001
EL1	MPO	ii. 5 ug/m	0.2469	_	0.0132	<.0001	ii. 5 ug/m	0.6161	_	0.0522	<.0001
EL1	MPO	iii. 20 ug	0.0908	0.0132	_	<.0001	iii. 20 ug	0.2917	0.0522	_	<.0001
EL1	MPO	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
EL1	SOD3	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	0.0003	<.0001	<.0001
EL1	SOD3	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	0.0003	_	<.0001	<.0001
EL1	SOD3	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001
EL1	SOD3	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
EL1	iNOS	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001
EL1	iNOS	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001
EL1	iNOS	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001
EL1	iNOS	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
FT33	CAT	i. 0 ug/ml	_	0.0015	<.0001	<.0001	i. 0 ug/ml	_	0.0067	<.0001	<.0001
FT33	CAT	ii. 5 ug/m	0.0015	_	0.0002	<.0001	ii. 5 ug/m	0.0067	_	0.0007	<.0001
FT33	CAT	iii. 20 ug	<.0001	0.0002	_	<.0001	iii. 20 ug	<.0001	0.0007	_	<.0001
FT33	CAT	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
FT33	GSR	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001
FT33	GSR	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001
FT33	GSR	iii. 20 ug	<.0001	<.0001	_	0.0026	iii. 20 ug	<.0001	<.0001	_	0.0109
FT33	GSR	iv. 100 ug	<.0001	<.0001	0.0026	_	iv. 100 ug	<.0001	<.0001	0.0109	_
FT33	GSTp1	i. 0 ug/ml	_	0.0037	0.0003	<.0001	i. 0 ug/ml	_	0.0155	0.0015	<.0001
FT33	GSTp1	ii. 5 ug/m	0.0037	_	0.0891	0.0006	ii. 5 ug/m	0.0155	_	0.2871	0.0027
FT33	GSTp1	iii. 20 ug	0.0003	0.0891	_	0.0079	iii. 20 ug	0.0015	0.2871	_	0.0325
FT33	GSTp1	iv. 100 ug	<.0001	0.0006	0.0079	_	iv. 100 ug	<.0001	0.0027	0.0325	_
FT33	MPO	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	0.0002	<.0001	<.0001
FT33	MPO	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	0.0002	_	<.0001	<.0001
FT33	MPO	iii. 20 ug	<.0001	<.0001	_	0.002	iii. 20 ug	<.0001	<.0001	_	0.0087
FT33	MPO	iv. 100 ug	<.0001	<.0001	0.002	_	iv. 100 ug	<.0001	<.0001	0.0087	_
FT33	SOD3	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	0.0003	<.0001	<.0001
FT33	SOD3	ii. 5 ug/m	<.0001	_	0.0593	<.0001	ii. 5 ug/m	0.0003	_	0.2036	<.0001
FT33	SOD3	iii. 20 ug	<.0001	0.0593	_	<.0001	iii. 20 ug	<.0001	0.2036	_	<.0001
FT33	SOD3	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
FT33	iNOS	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001
FT33	iNOS	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001

Cell_Line	Marker	R-Square
A2780	CAT	[illegible]
A2780	GSR	0.782237
A2780	GSTp1	0.946095
A2780	MPO	0.983782
A2780	SOD3	0.989886
A2780	iNOS	0.994602
EL1	CAT	0.549452
EL1	GSR	0.959807
EL1	GSTp1	0.729358
EL1	MPO	0.993123
EL1	SOD3	0.990868
EL1	iNOS	[illegible]
FT33	CAT	0.995613
FT33	GSR	0.997543
FT33	GSTp1	0.921688
FT33	MPO	0.989101
FT33	SOD3	0.990037
FT33	iNOS	[illegible]
NOE	CAT	0.995919
NOE	GSR	0.919797
NOE	GSTp1	[illegible]
NOE	MPO	0.986383
NOE	SOD3	0.992441
NOE	iNOS	[illegible]
SKOV3	CAT	0.917328
SKOV3	GSR	0.919797
SKOV3	GSTp1	0.993049
SKOV3	MPO	0.942852
SKOV3	SOD3	0.969597
SKOV3	iNOS	0.989288
TOV112	CAT	0.967695
TOV112	GSR	[illegible]
TOV112	GSTp1	0.936306
TOV112	MPO	0.993337
TOV112	SOD3	0.980254
TOV112	iNOS	0.992318

PCR

FT33	iNOS	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001		
FT33	iNOS	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_		
NOE	CAT	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001		
NOE	CAT	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	0.0002	<.0001		
NOE	CAT	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	0.0002	_	<.0001		
NOE	CAT	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_		
NOE	GSR	i. 0 ug/ml	_	0.1244	<.0001	<.0001	i. 0 ug/ml	_	0.3756	0.0004	0.0003		
NOE	GSR	ii. 5 ug/m	0.1244	_	0.0005	0.0003	ii. 5 ug/m	0.3756	_	0.0023	0.0015		
NOE	GSR	iii. 20 ug	<.0001	0.0005	_	0.7092	iii. 20 ug	0.0004	0.0023	_	0.979		
NOE	GSR	iv. 100 ug	<.0001	0.0003	0.7092	_	iv. 100 ug	0.0003	0.0015	0.979	_		
NOE	GSTp1	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	0.0001	<.0001	<.0001		
NOE	GSTp1	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	0.0001	_	<.0001	<.0001		
NOE	GSTp1	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001		
NOE	GSTp1	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_		
NOE	MPO	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001		
NOE	MPO	ii. 5 ug/m	<.0001	_	0.0005	<.0001	ii. 5 ug/m	<.0001	_	0.0021	<.0001		
NOE	MPO	iii. 20 ug	<.0001	0.0005	_	<.0001	iii. 20 ug	<.0001	0.0021	_	0.0004		
NOE	MPO	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	0.0004	_		
NOE	SOD3	i. 0 ug/ml	_	0.0004	<.0001	<.0001	i. 0 ug/ml	_	0.0017	<.0001	<.0001		
NOE	SOD3	ii. 5 ug/m	0.0004	_	<.0001	<.0001	ii. 5 ug/m	0.0017	_	0.0001	<.0001		
NOE	SOD3	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	0.0001	_	<.0001		
NOE	SOD3	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_		
NOE	iNOS	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001		
NOE	iNOS	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001		
NOE	iNOS	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001		
NOE	iNOS	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_		
SKOV3	CAT	i. 0 ug/ml	_	0.0304	0.002	<.0001	i. 0 ug/ml	_	0.1127	0.0084	<.0001		
SKOV3	CAT	ii. 5 ug/m	0.0304	_	0.0949	0.0002	ii. 5 ug/m	0.1127	_	0.3024	0.0009		
SKOV3	CAT	iii. 20 ug	0.002	0.0949	_	0.0017	iii. 20 ug	0.0084	0.3024	_	0.0076		
SKOV3	CAT	iv. 100 ug	<.0001	0.0002	0.0017	_	iv. 100 ug	<.0001	0.0009	0.0076	_		
SKOV3	GSR	i. 0 ug/ml	_	0.1244	<.0001	<.0001	i. 0 ug/ml	_	0.3756	0.0004	0.0003		
SKOV3	GSR	ii. 5 ug/m	0.1244	_	0.0005	0.0003	ii. 5 ug/m	0.3756	_	0.0023	0.0015		
SKOV3	GSR	iii. 20 ug	<.0001	0.0005	_	0.7092	iii. 20 ug	0.0004	0.0023	_	0.979		
SKOV3	GSR	iv. 100 ug	<.0001	0.0003	0.7092	_	iv. 100 ug	0.0003	0.0015	0.979	_		
SKOV3	GSTp1	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001		
SKOV3	GSTp1	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001		
SKOV3	GSTp1	iii. 20 ug	<.0001	<.0001	_	0.0004	iii. 20 ug	<.0001	<.0001	_	0.0016		
SKOV3	GSTp1	iv. 100 ug	<.0001	<.0001	0.0004	_	iv. 100 ug	<.0001	<.0001	0.0016	_		
SKOV3	MPO	i. 0 ug/ml	_	0.5259	0.0162	<.0001	i. 0 ug/ml	_	0.908	0.0634	<.0001		
SKOV3	MPO	ii. 5 ug/m	0.5259	_	0.0452	<.0001	ii. 5 ug/m	0.908	_	0.1607	<.0001		
SKOV3	MPO	iii. 20 ug	0.0162	0.0452	_	<.0001	iii. 20 ug	0.0634	0.1607	_	0.0004		
SKOV3	MPO	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	0.0004	_		
SKOV3	SOD3	i. 0 ug/ml	_	0.0413	0.0002	<.0001	i. 0 ug/ml	_	0.1483	0.001	<.0001		
SKOV3	SOD3	ii. 5 ug/m	0.0413	_	0.0042	<.0001	ii. 5 ug/m	0.1483	_	0.0177	<.0001		
SKOV3	SOD3	iii. 20 ug	0.0002	0.0042	_	<.0001	iii. 20 ug	0.001	0.0177	_	0.0001		
SKOV3	SOD3	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	0.0001	_		
SKOV3	iNOS	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001		
SKOV3	iNOS	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	0.0001	<.0001		
SKOV3	iNOS	iii. 20 ug	<.0001	<.0001	_	0.0005	iii. 20 ug	<.0001	0.0001	_	0.0022		
SKOV3	iNOS	iv. 100 ug	<.0001	<.0001	0.0005	_	iv. 100 ug	<.0001	<.0001	0.0022	_		
TOV112	CAT	i. 0 ug/ml	_	0.1929	0.0069	<.0001	i. 0 ug/ml	_	0.5213	0.0285	<.0001		
TOV112	CAT	ii. 5 ug/m	0.1929	_	0.0606	<.0001	ii. 5 ug/m	0.5213	_	0.2074	<.0001		
TOV112	CAT	iii. 20 ug	0.0069	0.0606	_	<.0001	iii. 20 ug	0.0285	0.2074	_	<.0001		
TOV112	CAT	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_		
TOV112	GSR	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001		
TOV112	GSR	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001		
TOV112	GSR	iii. 20 ug	<.0001	<.0001	_	0.0026	iii. 20 ug	<.0001	<.0001	_	0.0109		
TOV112	GSR	iv. 100 ug	<.0001	<.0001	0.0026	_	iv. 100 ug	<.0001	<.0001	0.0109	_		
TOV112	GSTp1	i. 0 ug/ml	_	0.1565	0.0081	<.0001	i. 0 ug/ml	_	0.4478	0.0332	<.0001		
TOV112	GSTp1	ii. 5 ug/m	0.1565	_	0.0895	<.0001	ii. 5 ug/m	0.4478	_	0.2882	0.0001		
TOV112	GSTp1	iii. 20 ug	0.0081	0.0895	_	0.0002	iii. 20 ug	0.0332	0.2882	_	0.0008		
TOV112	GSTp1	iv. 100 ug	<.0001	<.0001	0.0002	_	iv. 100 ug	<.0001	0.0001	0.0008	_		
TOV112	MPO	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001		
TOV112	MPO	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001		
TOV112	MPO	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	0.0004		
TOV112	MPO	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	0.0004	_		
TOV112	SOD3	i. 0 ug/ml	_	0.0035	0.0004	<.0001	i. 0 ug/ml	_	0.015	0.0019	<.0001		
TOV112	SOD3	ii. 5 ug/m	0.0035	_	0.1329	<.0001	ii. 5 ug/m	0.015	_	0.3954	<.0001		
TOV112	SOD3	iii. 20 ug	0.0004	0.1329	_	<.0001	iii. 20 ug	0.0019	0.3954	_	<.0001		
TOV112	SOD3	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_		
TOV112	iNOS	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001		
TOV112	iNOS	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001		
TOV112	iNOS	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	0.0004		
TOV112	iNOS	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	0.0004	_		

General linear model results ELISA

ELISA

Differences by exposure :

Cell_line	Marker	i/j	Nominal p-values i. 0 ug/ml	ii. 5 ug/m	iii. 20 ug	iv. 100 ug	Tokey-Kramer Adjusted i/j	i. 0 ug/ml	ii. 5 ug/m	iii. 20 ug	iv. 100 ug
EL-1	CAT	i. 0 ug/ml	_	0.0029	<.0001	<.0001	i. 0 ug/ml	_	0.0122	<.0001	<.0001
EL-1	CAT	ii. 5 ug/m	0.0029	_	<.0001	<.0001	ii. 5 ug/m	0.0122	_	<.0001	<.0001
EL-1	CAT	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001
EL-1	CAT	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
EL-1	GSR	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001
EL-1	GSR	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001
EL-1	GSR	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001
EL-1	GSR	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
EL-1	GST	i. 0 ug/ml	_	0.0053	0.0007	<.0001	i. 0 ug/ml	_	0.022	0.0033	<.0001
EL-1	GST	ii. 5 ug/m	0.0053	_	0.1761	<.0001	ii. 5 ug/m	0.022	_	0.4883	<.0001
EL-1	GST	iii. 20 ug	0.0007	0.1761	_	<.0001	iii. 20 ug	0.0033	0.4883	_	0.0003
EL-1	GST	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	0.0003	_
EL-1	MPO	i. 0 ug/ml	_	0.0048	0.0001	<.0001	i. 0 ug/ml	_	0.02	0.0006	<.0001
EL-1	MPO	ii. 5 ug/m	0.0048	_	0.0184	0.0009	ii. 5 ug/m	0.02	_	0.0713	0.0039
EL-1	MPO	iii. 20 ug	0.0001	0.0184	_	0.06	iii. 20 ug	0.0006	0.0713	_	0.2059
EL-1	MPO	iv. 100 ug	<.0001	0.0009	0.06	_	iv. 100 ug	<.0001	0.0039	0.2059	_
EL-1	NO2	i. 0 ug/ml	_	0.0002	<.0001	<.0001	i. 0 ug/ml	_	0.0008	<.0001	<.0001
EL-1	NO2	ii. 5 ug/m	0.0002	_	<.0001	<.0001	ii. 5 ug/m	0.0008	_	<.0001	<.0001
EL-1	NO2	iii. 20 ug	<.0001	<.0001	_	0.015	iii. 20 ug	<.0001	<.0001	_	0.0589
EL-1	NO2	iv. 100 ug	<.0001	<.0001	0.015	_	iv. 100 ug	<.0001	<.0001	0.0589	_
EL-1	SOD	i. 0 ug/ml	_	0.0004	<.0001	<.0001	i. 0 ug/ml	_	0.0016	<.0001	<.0001
EL-1	SOD	ii. 5 ug/m	0.0004	_	0.0008	<.0001	ii. 5 ug/m	0.0016	_	0.0037	0.0004
EL-1	SOD	iii. 20 ug	<.0001	0.0008	_	0.0745	iii. 20 ug	<.0001	0.0037	_	0.2475
EL-1	SOD	iv. 100 ug	<.0001	<.0001	0.0745	_	iv. 100 ug	<.0001	0.0004	0.2475	_
FT33	CAT	i. 0 ug/ml	_	0.0003	<.0001	<.0001	i. 0 ug/ml	_	0.0016	<.0001	<.0001
FT33	CAT	ii. 5 ug/m	0.0003	_	<.0001	<.0001	ii. 5 ug/m	0.0016	_	<.0001	<.0001
FT33	CAT	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001
FT33	CAT	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
FT33	GSR	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001
FT33	GSR	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001
FT33	GSR	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001
FT33	GSR	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
FT33	GST	i. 0 ug/ml	_	0.0001	<.0001	<.0001	i. 0 ug/ml	_	0.0006	<.0001	<.0001
FT33	GST	ii. 5 ug/m	0.0001	_	0.0002	<.0001	ii. 5 ug/m	0.0006	_	0.0008	<.0001
FT33	GST	iii. 20 ug	<.0001	0.0002	_	<.0001	iii. 20 ug	<.0001	0.0008	_	<.0001
FT33	GST	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
FT33	MPO	i. 0 ug/ml	_	0.003	0.4263	0.0041	i. 0 ug/ml	_	0.0129	0.8352	0.0173
FT33	MPO	ii. 5 ug/m	0.003	_	0.01	0.8308	ii. 5 ug/m	0.0129	_	0.0405	0.9959
FT33	MPO	iii. 20 ug	0.4263	0.01	_	0.014	iii. 20 ug	0.8352	0.0405	_	0.0552
FT33	MPO	iv. 100 ug	0.0041	0.8308	0.014	_	iv. 100 ug	0.0173	0.9959	0.0552	_
FT33	NO2	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001
FT33	NO2	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001
FT33	NO2	iii. 20 ug	<.0001	<.0001	_	0.0366	iii. 20 ug	<.0001	<.0001	_	0.1334
FT33	NO2	iv. 100 ug	<.0001	<.0001	0.0366	_	iv. 100 ug	<.0001	<.0001	0.1334	_
FT33	SOD	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001
FT33	SOD	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001
FT33	SOD	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	0.0001
FT33	SOD	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	0.0001	_
A2780	CAT	i. 0 ug/ml	_	0.1307	<.0001	<.0001	i. 0 ug/ml	_	0.3904	<.0001	<.0001
A2780	CAT	ii. 5 ug/m	0.1307	_	<.0001	<.0001	ii. 5 ug/m	0.3904	_	0.0004	<.0001
A2780	CAT	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	0.0004	_	<.0001
A2780	CAT	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
A2780	GSR	i. 0 ug/ml	_	0.0002	<.0001	<.0001	i. 0 ug/ml	_	0.0011	<.0001	<.0001
A2780	GSR	ii. 5 ug/m	0.0002	_	<.0001	<.0001	ii. 5 ug/m	0.0011	_	<.0001	<.0001
A2780	GSR	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001
A2780	GSR	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
A2780	GST	i. 0 ug/ml	_	0.033	<.0001	<.0001	i. 0 ug/ml	_	0.1216	0.0004	<.0001
A2780	GST	ii. 5 ug/m	0.033	_	0.0018	<.0001	ii. 5 ug/m	0.1216	_	0.0079	<.0001
A2780	GST	iii. 20 ug	<.0001	0.0018	_	<.0001	iii. 20 ug	0.0004	0.0079	_	0.0005
A2780	GST	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	0.0005	_
A2780	MPO	i. 0 ug/ml	_	0.0002	<.0001	<.0001	i. 0 ug/ml	_	0.0009	<.0001	<.0001
A2780	MPO	ii. 5 ug/m	0.0002	_	0.0032	0.0002	ii. 5 ug/m	0.0009	_	0.0136	0.0008
A2780	MPO	iii. 20 ug	<.0001	0.0032	_	0.0457	iii. 20 ug	<.0001	0.0136	_	0.1624
A2780	MPO	iv. 100 ug	<.0001	0.0002	0.0457	_	iv. 100 ug	<.0001	0.0008	0.1624	_
A2780	NO2	i. 0 ug/ml	_	0.0436	0.0015	<.0001	i. 0 ug/ml	_	0.1557	0.0065	<.0001
A2780	NO2	ii. 5 ug/m	0.0436	_	0.0477	0.0001	ii. 5 ug/m	0.1557	_	0.1687	0.0006
A2780	NO2	iii. 20 ug	0.0015	0.0477	_	0.002	iii. 20 ug	0.0065	0.1687	_	0.0086
A2780	NO2	iv. 100 ug	<.0001	0.0001	0.002	_	iv. 100 ug	<.0001	0.0006	0.0086	_
A2780	SOD	i. 0 ug/ml	_	0.0489	<.0001	<.0001	i. 0 ug/ml	_	0.1724	<.0001	<.0001
A2780	SOD	ii. 5 ug/m	0.0489	_	0.0001	<.0001	ii. 5 ug/m	0.1724	_	0.0006	<.0001

Model fit

Cell_line	Marker	R-Square
EL-1	CAT	0.991514
EL-1	GSR	[illegible]
EL-1	GST	0.956082
EL-1	MPO	0.919367
EL-1	NO2	0.986623
EL-1	SOD	0.962855
FT33	CAT	0.995747
FT33	GSR	[illegible]
FT33	GST	0.990876
FT33	MPO	0.775049
FT33	NO2	0.993174
FT33	SOD	0.994496
A2780	CAT	0.983712
A2780	GSR	[illegible]
A2780	GST	0.967063
A2780	MPO	0.960296
A2780	NO2	0.920775
A2780	SOD	0.96559
NOE	CAT	0.994361
NOE	GSR	[illegible]
NOE	GST	0.956404
NOE	MPO	[illegible]
NOE	NO2	[illegible]
NOE	SOD	0.983272
SKOV-3	CAT	0.977149
SKOV-3	GSR	[illegible]
SKOV-3	GST	0.97881
SKOV-3	MPO	[illegible]
SKOV-3	NO2	[illegible]
SKOV-3	SOD	0.978235
TOV-112	CAT	0.991059
TOV-112	GSR	[illegible]
TOV-112	GST	0.975344
TOV-112	MPO	0.990375
TOV-112	NO2	0.970334
TOV-112	SOD	0.988387

ELISA

A2780	SOD	iii. 20 ug	<.0001	0.0001	_	0.0035	iii. 20 ug	<.0001	0.0006	_	0.0149
A2780	SOD	iv. 100 ug	<.0001	<.0001	0.0035	_	iv. 100 ug	<.0001	<.0001	0.0149	_
NOE	CAT	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	0.0003	<.0001	<.0001
NOE	CAT	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	0.0003	_	<.0001	<.0001
NOE	CAT	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001
NOE	CAT	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
NOE	GSR	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001
NOE	GSR	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001
NOE	GSR	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001
NOE	GSR	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
NOE	GST	i. 0 ug/ml	_	0.0086	0.0001	<.0001	i. 0 ug/ml	_	0.0349	0.0005	<.0001
NOE	GST	ii. 5 ug/m	0.0086	_	0.0065	<.0001	ii. 5 ug/m	0.0349	_	0.0268	<.0001
NOE	GST	iii. 20 ug	0.0001	0.0065	_	0.0005	iii. 20 ug	0.0005	0.0268	_	0.0024
NOE	GST	iv. 100 ug	<.0001	<.0001	0.0005	_	iv. 100 ug	<.0001	<.0001	0.0024	_
NOE	MPO	i. 0 ug/ml	_	0.0152	0.0907	0.0579	i. 0 ug/ml	_	0.0597	0.2915	0.1994
NOE	MPO	ii. 5 ug/m	0.0152	_	0.282	0.4129	ii. 5 ug/m	0.0597	_	0.6697	0.8231
NOE	MPO	iii. 20 ug	0.0907	0.282	_	0.7793	iii. 20 ug	0.2915	0.6697	_	0.9909
NOE	MPO	iv. 100 ug	0.0579	0.4129	0.7793	_	iv. 100 ug	0.1994	0.8231	0.9909	_
NOE	NO2	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001
NOE	NO2	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001
NOE	NO2	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001
NOE	NO2	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
NOE	SOD	i. 0 ug/ml	_	0.0016	<.0001	<.0001	i. 0 ug/ml	_	0.0071	<.0001	<.0001
NOE	SOD	ii. 5 ug/m	0.0016	_	<.0001	<.0001	ii. 5 ug/m	0.0071	_	<.0001	<.0001
NOE	SOD	iii. 20 ug	<.0001	<.0001	_	0.0099	iii. 20 ug	<.0001	<.0001	_	0.0401
NOE	SOD	iv. 100 ug	<.0001	<.0001	0.0099	_	iv. 100 ug	<.0001	<.0001	0.0401	_
SKOV-3	CAT	i. 0 ug/ml	_	0.0038	0.0001	<.0001	i. 0 ug/ml	_	0.0162	0.0006	<.0001
SKOV-3	CAT	ii. 5 ug/m	0.0038	_	0.0241	<.0001	ii. 5 ug/m	0.0162	_	0.0913	<.0001
SKOV-3	CAT	iii. 20 ug	0.0001	0.0241	_	<.0001	iii. 20 ug	0.0006	0.0913	_	<.0001
SKOV-3	CAT	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
SKOV-3	GSR	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001
SKOV-3	GSR	ii. 5 ug/m	<.0001	_	0.0002	<.0001	ii. 5 ug/m	<.0001	_	0.0008	<.0001
SKOV-3	GSR	iii. 20 ug	<.0001	0.0002	_	<.0001	iii. 20 ug	<.0001	0.0008	_	<.0001
SKOV-3	GSR	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
SKOV-3	GST	i. 0 ug/ml	_	0.0042	<.0001	<.0001	i. 0 ug/ml	_	0.0175	<.0001	<.0001
SKOV-3	GST	ii. 5 ug/m	0.0042	_	0.0011	<.0001	ii. 5 ug/m	0.0175	_	0.0047	<.0001
SKOV-3	GST	iii. 20 ug	<.0001	0.0011	_	<.0001	iii. 20 ug	<.0001	0.0047	_	<.0001
SKOV-3	GST	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
SKOV-3	MPO	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001
SKOV-3	MPO	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001
SKOV-3	MPO	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001
SKOV-3	MPO	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
SKOV-3	NO2	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	0.0004	<.0001	<.0001
SKOV-3	NO2	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	0.0004	_	<.0001	<.0001
SKOV-3	NO2	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001
SKOV-3	NO2	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
SKOV-3	SOD	i. 0 ug/ml	_	0.0006	<.0001	<.0001	i. 0 ug/ml	_	0.0027	<.0001	<.0001
SKOV-3	SOD	ii. 5 ug/m	0.0006	_	0.0002	<.0001	ii. 5 ug/m	0.0027	_	0.001	<.0001
SKOV-3	SOD	iii. 20 ug	<.0001	0.0002	_	0.0003	iii. 20 ug	<.0001	0.001	_	0.0013
SKOV-3	SOD	iv. 100 ug	<.0001	<.0001	0.0003	_	iv. 100 ug	<.0001	<.0001	0.0013	_
TOV-112	CAT	i. 0 ug/ml	_	0.0004	<.0001	<.0001	i. 0 ug/ml	_	0.0018	<.0001	<.0001
TOV-112	CAT	ii. 5 ug/m	0.0004	_	<.0001	<.0001	ii. 5 ug/m	0.0018	_	<.0001	<.0001
TOV-112	CAT	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001
TOV-112	CAT	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
TOV-112	GSR	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001
TOV-112	GSR	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	<.0001	_	<.0001	<.0001
TOV-112	GSR	iii. 20 ug	<.0001	<.0001	_	<.0001	iii. 20 ug	<.0001	<.0001	_	<.0001
TOV-112	GSR	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
TOV-112	GST	i. 0 ug/ml	_	0.0051	<.0001	<.0001	i. 0 ug/ml	_	0.0212	<.0001	<.0001
TOV-112	GST	ii. 5 ug/m	0.0051	_	0.0002	<.0001	ii. 5 ug/m	0.0212	_	0.001	<.0001
TOV-112	GST	iii. 20 ug	<.0001	0.0002	_	0.0002	iii. 20 ug	<.0001	0.001	_	0.001
TOV-112	GST	iv. 100 ug	<.0001	<.0001	0.0002	_	iv. 100 ug	<.0001	<.0001	0.001	_
TOV-112	MPO	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	<.0001	<.0001	<.0001
TOV-112	MPO	ii. 5 ug/m	<.0001	_	0.0006	<.0001	ii. 5 ug/m	<.0001	_	0.0025	<.0001
TOV-112	MPO	iii. 20 ug	<.0001	0.0006	_	<.0001	iii. 20 ug	<.0001	0.0025	_	<.0001
TOV-112	MPO	iv. 100 ug	<.0001	<.0001	<.0001	_	iv. 100 ug	<.0001	<.0001	<.0001	_
TOV-112	NO2	i. 0 ug/ml	_	<.0001	0.0007	<.0001	i. 0 ug/ml	_	<.0001	0.0029	<.0001
TOV-112	NO2	ii. 5 ug/m	<.0001	_	0.0057	0.0002	ii. 5 ug/m	<.0001	_	0.0239	0.0008
TOV-112	NO2	iii. 20 ug	0.0007	0.0057	_	<.0001	iii. 20 ug	0.0029	0.0239	_	<.0001
TOV-112	NO2	iv. 100 ug	<.0001	0.0002	<.0001	_	iv. 100 ug	<.0001	0.0008	<.0001	_
TOV-112	SOD	i. 0 ug/ml	_	<.0001	<.0001	<.0001	i. 0 ug/ml	_	0.0003	<.0001	<.0001
TOV-112	SOD	ii. 5 ug/m	<.0001	_	<.0001	<.0001	ii. 5 ug/m	0.0003	_	<.0001	<.0001
TOV-112	SOD	iii. 20 ug	<.0001	<.0001	_	0.0006	iii. 20 ug	<.0001	<.0001	_	0.0025
TOV-112	SOD	iv. 100 ug	<.0001	<.0001	0.0006	_	iv. 100 ug	<.0001	<.0001	0.0025	_

Exhibit I

Personal Information

Black n' Red

Date	Experiment	Pages
9/26/2017	Prepareration	38
9/26/2017	Thawing cells	39
9/29/2017	Subculture cells	39-40
10/6/2017	Seeded cells	40
10/7/2017	Treat cells with Talc	40
10/10/2017	Collect cells&RNA extraction	41-43
10/11/2017	Run RT-PCR b-actin with Standard&sample	44-47
10/13/2017	Run RT-PCR GSR with Standard&sample	48-49
10/16/2017	Run RT-PCR iNOS with Standard&sample	50-51
10/16/2017	Run RT-PCR GPX1 with Standard&sample	52-53
10/17/2017	Run RT-PCR SOD3 with Standard&sample	54-55
10/18/2018	Run RT-PCR Catalase with Standard&samples	56-57
10/18/2017	Run RT-PCR MPO with Standard&sample	58-59
10/19/2017	Run RT-PCR GSTp1 with Standard&sample	60-61
10/20/2017	Talc EOC Poster	62-63

DEPOSITION EXHIBIT 9
Saed
1-23-19 LF
PENGAD 800-631-6989

Use this page as a quick reference to the book's contents during use and for archiving purposes

www.BlacknRed.com

9/26/2017

- Cell lines

SKOV-3	ATCC
A2780	Sigma Aldrich, St. Louis MO
TOV112	A kind gift from Genshong Wu at Wayne State Ur

EL-1 / macrophages

Normal Ovarian epithelial Cell Biologic, Chicago, IL

- Fetal bovine serum (FBS, Innovative Research, Novi, MI)
Penicilin/streptomycin (Fisher Scientific)

- Talc (Fisher # T4-500 Lot # 166820)

Seeded Cells for PCR

9/26/2017

— Thawing Cells

	Media
EL-1 (Macrophages)	IMDM (10% FBS, 1% PS, 1ml H-T, 2μL
Normal Ovarian Epithelial	Complete Human Epithelial Cell medium k (Cell Biologics)
Skov-3	McCoy's 5A (10% FBS, 1% PS)
TOV112	Medium 199: MCDB 105 (1:1) + 10% FBS +
AL780	RPMI-1640 (10% FBS + 1% PS)

75 cm² flask + 15 mL medium

9/29/2017

— Subculture cells

※ Check ~~the~~ under microscop cells are 70~80% full.

① Sork out old medium
② Wash with 10mL PBS
③ gently remove PBS
④ pipet trypsin-EDTA 2mL onto the washed Cells mondarger
※ Normal Ovarian Epithelial use trypsn from ScienCell
⑤ 37°C incubator 1~5 minutes (Skov-3 longer)
⑥ Check under microscope
⑦ Add fresh medium 8mL to inactive trypsin. Then mix
⑧ Take 2mL to a new 100mm dish
⑨ Add 8mL Fresh medium to 100mm dish
⑩ Incubate the cells

※ One time ~~treat~~ ~~one cell~~ One Cell line.

10/3/2017

- Subculture Cells

  2mL Cells + 8mL medium 100 mm dish

  Cells doubled in one day

10/6/2017

- Subculture Cells

- Seeded Cells for talc treat
  1×10^6 cells/dish 60mm dish + 5mL medium

10/7/2017

- Treat cells with talc
  Prepare talc
  100mg talc + 10ml DMSO → mix 10 mg/mL = 10^4 µg/mL

- sterilization under UV light to avoid endotoxin and microbial Contamination

$(x_1)(10^4\ \mu g/mL) = (5mL)(20\ \mu g/mL) \rightarrow x_1 = 10\ \mu L$

$(x_2)(10^4\ \mu g/mL) = (5mL)(100\ \mu g/mL) \rightarrow x_2 = 50\ \mu L$

$(x_3)(10^4\ \mu g/mL) = (5mL)(1000\ \mu g/mL) \rightarrow x_3 = 500\ \mu L$

ini

10/10/2017
After 72 hours treatment

— Collect cells

Cell Collection Protocal

Put on gloves and spray with 70% ethanol
Remove cell culture dish from incubator
Observe cells under microscope.
Move the dishes to your work bench, does not need to be done in the hood.
Collect media and place in labeled 15ml tube for freezing,
Add 10 ml PBS
Using a cell scraper, scrape the bottom of the dish and rotate it to ensure scraping of entire bottom
Using a 10ml pipet, remove the PBS and cell mixture and place into the 15ml conical centrifugation tube that corresponds to the dish, 1ml for RNA, 2ml for DNA,8 ml for protein assay.
Close and centrifuge all tubes, 5 minutes at 1800rpm (slower speed keeps cells from breaking).
Place another paper towel by sink, dump PBS from all tubes into sink and place tubes upside down to drain them. Cells will be collected at the bottom. Place all tubes in Styrofoam holder and place in -80°C freezer.

— RNA Extraction
RNeasy Mini Kit (Qiagen Cat # 74106) (go to pg 42. 43)

— Detect Concentration of RNA by Nanodrop
(Thermo Fisher Scientific)
(go pg 43)

— cDNA Synthesis via Reverse Transcription — VILO kit
Life Technolog
(go pg 43)

RNA Extraction

RNeasy Mini Kit (Qiagen cat # 74106)

Important Notes before starting: WORK IN THE HOOD

- β-Mercaptoethanol (β-ME) can be added to Buffer RLT (lysis buffer) before use. β-ME is toxic; dispense in a fume hood and wear appropriate protective clothing. Add 10 µl β-ME per 1 ml Buffer RLT. Buffer RLT is stable for **one month** after addition of β-ME.
- Buffer RPE is supplied as a concentrate. Before using for the first time, add ethanol as indicated on the bottle. Be sure to **mark the lid with a X** to show that the working solution has been prepared.

Buffer RW1 and Buffer RLT are hazardous.

- **Buffer RLT+ β-ME should be disposed of in the jar in the hood.**
- **Buffer RWI should be disposed of in the jar in the hood.**

Preparation of the Buffer RLT

- In a labeled 15ml centrifugation tube, add 10µl β-ME for every 1 ml Buffer RLT.

Preparation of your samples

1. Add 350 µl of the Buffer RLT + β-ME solution to each of your sample tubes.
   a. if you have a lot of cells, you will need to add 600 µl of Buffer RLT + β-ME solution to each tube ***also add equal volume of ethanol)
2. Add 350 µl of 70% ethanol to each tube and pipet to mix
3. Transfer the entire sample to its corresponding mini spin column
   a. Close columns and place them into the small centrifuge.
   b. Centrifuge the tubes for 15 seconds at 13,000 rpm
4. Dump the flow through into hazardous waste jar **in the hood**.
5. Add 700µl of the Buffer RW1 to the RNeasy column
   a. Centrifuge 15 seconds at 13,000 rpm
6. Dump the flow through into hazardous waste jar **in the hood**
7. Add 500µl of Buffer RPE onto each RNeasy column
   a. Centrifuge 15 seconds at 13,000 rpm
8. Dump the flow through into waste jar
9. Add 500µl Buffer RPE to each column again
   a. Centrifuge 2 minutes at 13,000 rpm to dry the silica gel membrane
10. Dump the flow through in waste jar, centrifuge for one minute more
11. Remove columns from collection tubes and place in corresponding 1.5ml centrifuge tube
12. Add 50µl of RNase-free water to each column, onto the center of the silica-gel membrane without touching the sides of the column (water dissolves RNA).
    a. Allow to stand for 1 minute
    b. Centrifuge columns for 1 minute at 13,000 rpm, LID MUST BE ON CENTRIFUGE
13. Collect flow through from the collection tube and place back into the column on the center of the membrane, allow to stand for 1 minute
    a. Centrifuge columns again for 1 minute at 13,000 rpm, LID MUST BE ON CENTRIFUGE
14. Remove and dispose of columns
15. Place your microcentrifuge tubes containing RNA on ice
    a. Detect concentration of RNA
    b. Good quality RNA has a A260/A280 of 2.0

NEED TO MEASURE RNA EACH TIME YOU GO TO MAKE cDNA

cDNA Synthesis via Reverse Transcription

You will need:
Ice
Thaw, on ice:
RNA
VILO MasterMix
RNase-free water

You must detect the concentration of your RNA. After doing this, you can calculate the volume needed to get for a 1 µg reaction.
i.e. – If your RNA concentration is 0.9 ug/ul then:
(x ul)(0.9 ug/ul) = 1 ug solve for x

For a single reaction, combine the following components in a sterile PCR tube on ice.

	1 µg RNA
Component	**Volume/reaction**
VILO MasterMix	4 µl
Template RNA	Variable up to 1 µg
RNase-free Water	Variable
Total Volume:	20 µl

The total amount in each tube should equal **20 ul**, hence the variable volume of water.

- Add 4 ul VILO MasterMix to each tube, volume of RNA calculated, volume of water calculated, and gently mix.
- Place the tubes in a rack and the rack into a 25°C water bath for 10 minutes.
- Place the rack into a 42°C water bath for 60 minutes.
- Then, place racked tubes into 85°C water bath for 5 minutes to terminate the reaction.
- Place samples on ice for a few minutes.
- Centrifuge cDNA.
- Place into -80°C freezer for storage or continue on.

Do 0.2ug Reaction

Sample	Concentration ug/ul RNA	ul RNA for 0.2 ug in 1.5 ug rxn	µl Water
SKOV unt 72 hr	0.0521	3.8	20.2
SKOV talc 20ug/ml 72 hr	0.0431	4.6	19.4
A2780 unt 72 hr	0.0976	2.0	22.0
A2780 talc 20 ug/ml 72 hr	0.1067	1.9	22.1
EL1 72 hr	0.0067	24.0	0.0
EL1 talc 20ug/ml 72 hr	0.0146	11.0	13.0
SKOV talc 100ug/ml 72 hr	0.086	2.3	21.7
SKOV talc 1000ug/ml 72 hr	0.0592	3.4	20.6
A2780 talc100ug/ml 72 hr	0.0289	6.9	17.1
A2780 talc 1000ug/ml72 hr	0.0335	6.0	18.0
EL1 talc 100ug/ml 72 hr	0.0104	15.5	8.5
EL1 talc1000ug/ml 72 hr	0.0128	12.6	11.4
Normal OV Epi 72 hr	0.0433	4.6	19.4
Normal OV Epi talc 20ug/ml72 hr	0.0385	5.2	18.8
Normal Ov Epi talc 100ug/ml72 hr	0.0357	5.6	18.4
Normal Ov Epi talc 1000ug/ml72 hr	0.0667	3.0	21.0

0.2µg RNA was obtained from each sample following dilution as described by this table

cDNA (30ng) prepared

1/2

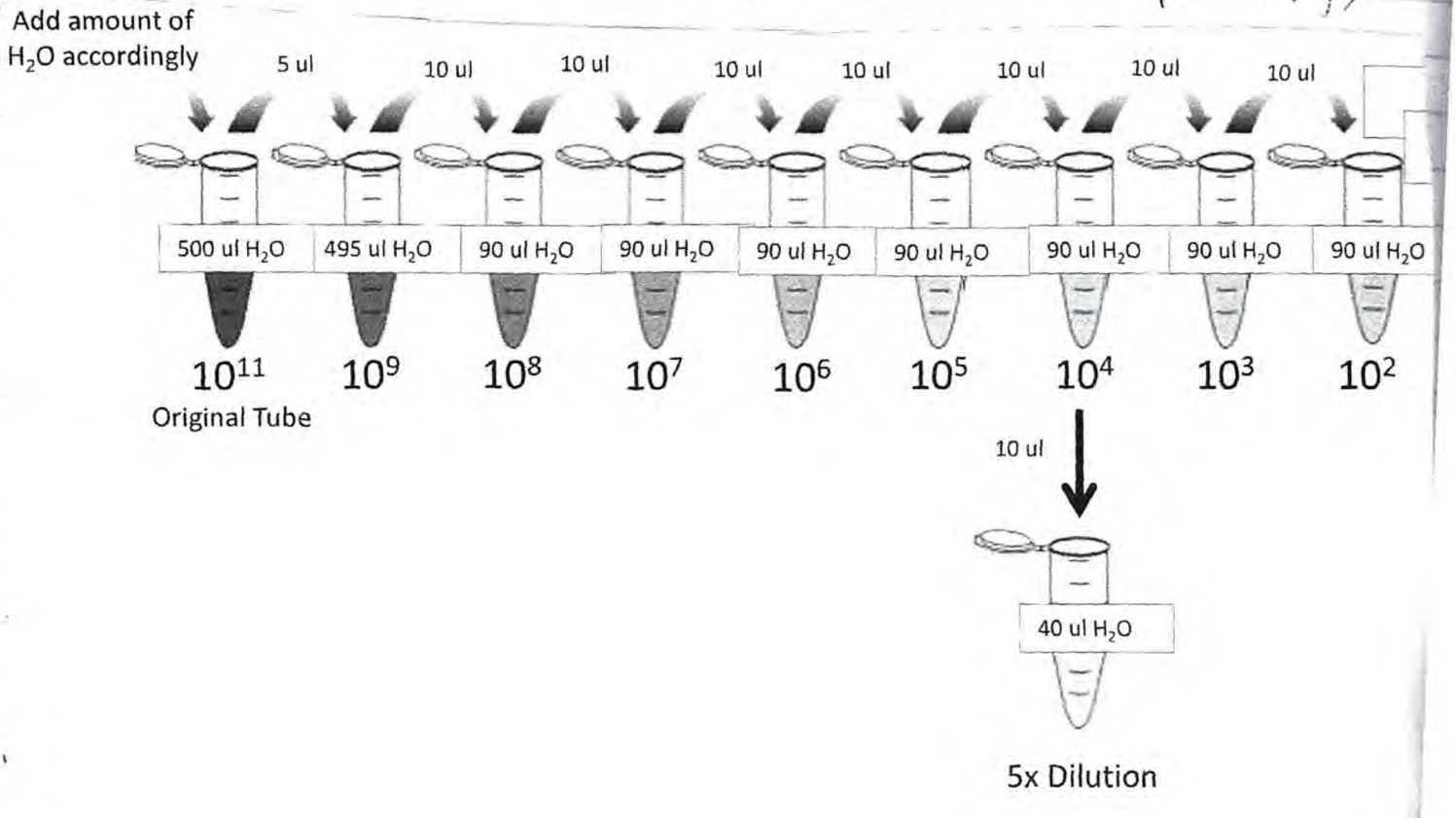
10/11/2017 Real-time PCR for β-actin

β-actin – Standard

– Standards come desiccated

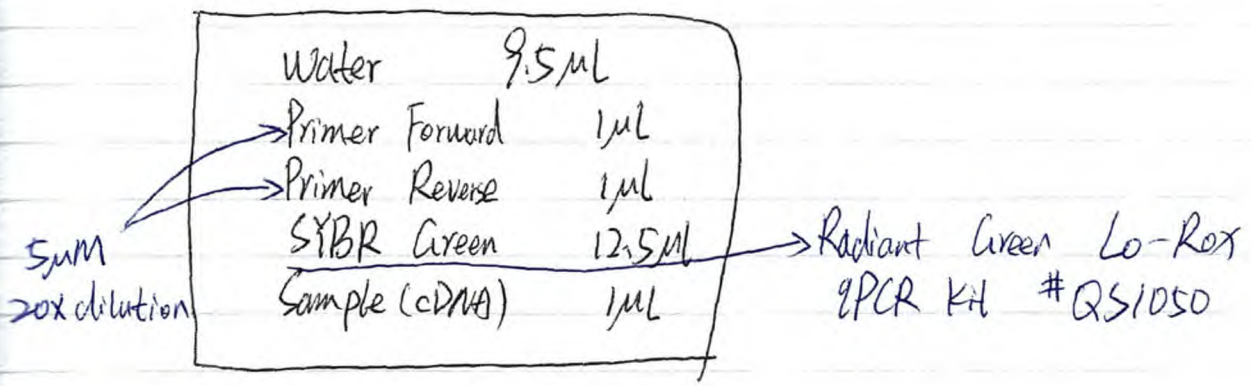
- Reconstitute the standard using TE buffer
- The volume of TE buffer is on the product sheet
  ① You will add TE buffer such that the concentration will be 100μM
  - Mix well
  ② In a new 1.5mL microtube, add 5μl of standard to each tube
  - Calculate the number of tubes needed by dividing the volume of TE buffer you added by 5
  ③ put tubes into the concentrator machine for 2minutes – Lids open
  ④ Close tubes, label the lid with the type of standard and date
  - The box should state that user add 500μl of PCR water to get a standard that is 10^{11}

– Serial Dilution of Standard (place samples on ice after mixing)



Run β-actin with samples

— Do 25µl reaction



— Calculating Master mix for samples

20 samples X 3 (triplicated) + 1 blank = 61
61 X 1.17 extra = 71.37

— Master mix calculation

Water = 9.5 X 71.37 = 678.015µL = 678µL
primer = 1 X 71.37 = 71.4µL
SYBR Green = 12.5 X 71.37 = 892.125 = 892.1

— Mix, then take out 80.6µL of this mix ⟶ 1.5mL tube/~~per~~ sample
24 X 3 X 1.12 extra = 80.6µL

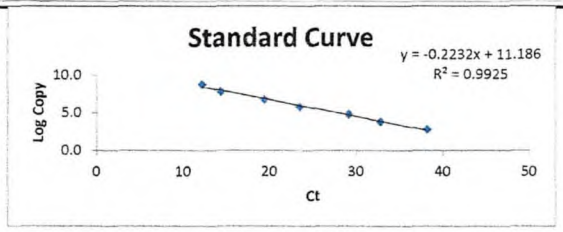
— Add 3.4µl Sample to 1.5mL tube containing master mix
1 X 3 X 1.12 = 1.4µL

— Mix well add 25µL ⟶ PCR tube
3 total per sample

β-actin Gene information

Gene of Interest	B-actin		
		Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	79	bases	
Mass in Daltons	2.41E+04	Da	= number bases x avg. mass/base
Mass in grams	4.00E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	4.00E-14	ug	= above / 10E-6
Mass in ng	4.00E-11	ng/copy	= above x 10E3

Copy #	Ct	Log Copy #
610000000	12.29	8.8
61000000	13.15	7.8
6099999.5	16.12	6.8
610000	20.69	5.8
61000	24.74	4.8
6100	28.15	3.8
610	31.71	2.8



Oligonucleotide primers and cycling condition

Accession Number	Gene	Sense (5'-3')	Antisense (3'-5')	Amplicon (bp)	Annealing Time (sec) and Temperature (°C)
NM_001101	β-actin	ATGACTTAGTTGCGTTACAC	AATAAAGCCATGCCAATCTC	79	10, 64
NM_001752	CAT	GGTTGAACAGATAGCCTTC	CGGTGAGTGTCAGGATAG	105	10, 63
NM_000637	GSR	TCACCAAGTCCCATATAGAAATC	TGTGGCGATCAGGATGTG	116	10, 63
NM_000581	GPX1	GGACTACACCCAGATGAAC	TTCTCCTGATGCCCAAAC	96	10, 61
NM_000852	GSTp1	TACCAGTCCAATACCATC	GTAGATGAGGGAGATGTA	138	10, 57
NM_000250	MPO	CACTTGTATCCTCTGGTTCTTCAT	TCTATATGCTTCTCACGCCTAGTA	79	60, 63
NM_000625	NOS2	GAGGACCACATCTACCAGGAGGAG	CCAGGCAGGCGGGAATAGG	89	30, 59
NM_003102	SOD3	GTGTTCCTGCCTGCTCCT	TCCGCCGAGTCAGAGTTG	84	60, 64

An initial cycle was performed at 95°C followed by 35 cycles of denaturation at 95°C for 15 seconds, annealing temperature and time per the table, followed by extension cycle at 72°C for 30 seconds.

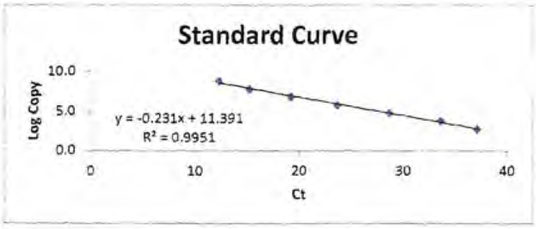
10/13/2017

Run Real-time PCR - GSR with standard & samples

Gene of Interest	GSR		
		Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	103	bases	
Mass in Daltons	3.14E+04	Da	= number bases x avg. mass/base
Mass in grams	5.22E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	5.22E-14	ug	= above / 10E-6
Mass in ng	5.22E-11	ng/copy	= above x 10E3

Gene information

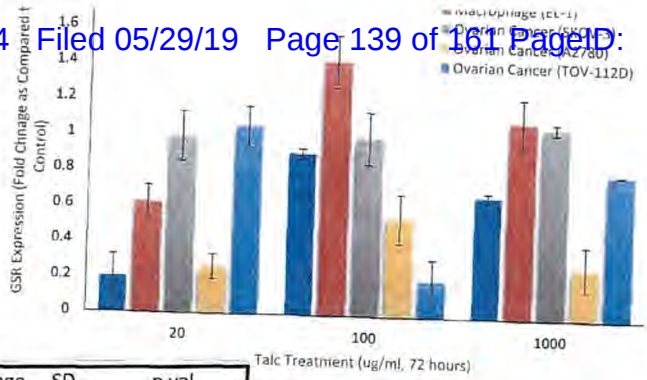
Copy #	Ct	Log Copy #
607000000	12.29	8.8
60700000	13.15	7.8
6070000	16.12	6.8
607000	20.69	5.8
60700	24.74	4.8
6070	28.15	3.8
607	31.71	2.8



Standard Curve

Data

Normal Ov Epithelial Cells	fg/ul cDNA	Fold Change	Average	SD	p val
Normal Ov Epithelial -Control	4.46428128	4.252409179			
	4.040537078				
	5.649374711				
Normal Ov Epithelial 20 ug/ml Talc	3.436513604	-0.191866667	0.200031	0.122962	0.3818
	4.733285555	0.11308328			
	5.472758654	0.286978375			
Normal Ov Epithelial 100 ug/ml Talc	8.010433347	0.883740019	0.904084	0.028771	0.0493
	8.183455957	0.924428156			
	6.552998884	0.541008545			
Normal Ov Epithelial 1000 ug/ml Talc	7.045842037	0.656905942	0.674378	0.02471	0.0633
	7.629372716	0.794129491			
	7.194442969	0.691851058			
EL-1 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
EL1 Control DMSO (5 ug/ml volume)	57.54265341	56.14675379			
	56.08810465				
	54.80950331				
EL1 20 ug/ml Talc	94.65256367	0.685806521	0.619541	0.093714	0.05
	103.7557756	0.847938992			
	87.21138337	0.553275612			
EL1 100 ug/ml Talc	130.0248721	1.315803912	1.418008	0.144539	0.05
	117.4033866	1.09100934			
	141.5017433	1.520212368			
EL1 1000 ug/ml Talc	125	1.226308585	1.095698	0.133677	0.0042
	110	0.959151555			
	118	1.101635305			



SKOV-3 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
SKOV control for 20 ug/ml Talc	114.1745767	120.5460773			
	127.061285				
	120.4023703				
SKOV-3 Control for 100 ug/ml Talc	101.1965313	103.1239404			
	105.0513496				
	74.08540446				
SKOV-3 20 ug/ml	193.1882215	0.602608942	0.988734	0.138282	0.0825
	227.9470905	0.890954028			
	251.5211034	1.08651421			
SKOV-3 100 ug/ml	228.5877349	1.216631114	0.993998	0.146443	0.043
	216.3075686	1.09754949			
	194.9503956	0.890447502			
SKOV-3 control for 1000 ug/ml Talc	8.767536762	8.745966216			
	12.50147198				
	8.72439567				
SKOV-3 1000 ug/ml Talc	18.23385621	1.084830396	1.063699	0.029884	0.0112
	11.80474342	0.349735768			
	17.86422909	1.042567813			

A2780 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
A2780 control for 20 ug/ml Talc	140.1662906	131.4102463			
	133.6702915				
	129.1502011				
A2780 Control for 100 ug/ml Talc	59.80237268	58.6912448			
	59.17958401				
	57.09177772				
A2780 20 ug/ml	217.2079848	0.652899914	0.26082	0.068898	0.0766
	172.0867487	0.309538286			
	159.2825972	0.212101808			
A2780 100 ug/ml	96.49799078	0.644163301	0.546833	0.137646	0.1088
	85.07310653	0.449502508			
	114.628098	0.953069805			
A2780 control for 1000 ug/ml Talc	3.268388429	5.483649208			
	7.698909987				
	0.370810318				
A2780 1000 ug/ml Talc	5.048597924	-0.079336089	0.282275	0.124705	0.5365
	6.547999324	0.194095223			
	7.515090464	0.370454269			

TOV112 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	72.41291598	72.18860045			
	71.52391916				
	72.62896621				
TOV112 20 ug/ml Talc	155.3169283	1.151543697	1.051945	0.108838	0.0035
	139.7405248	0.935769968			
	149.3235813	1.068520242			
TOV112 control for 100 talc	5.996678626	7.837259573			
	7.53579081				
	9.979309283				
TOV112 100 ug/ml Talc	10.08078007	0.286263391	0.203338	0.117274	0.4114
	8.78096925	0.120413222			
	7.925913775	0.011311888			
TOV112 Control for 1000 tov	7.753431026	7.785682592			
	7.825356753				
	7.778259997				
TOV112D 1000 Talc 48 hr	14.05672982	0.805458886	0.806772	0.001857	0.0026
	14.07717885	0.808085378			

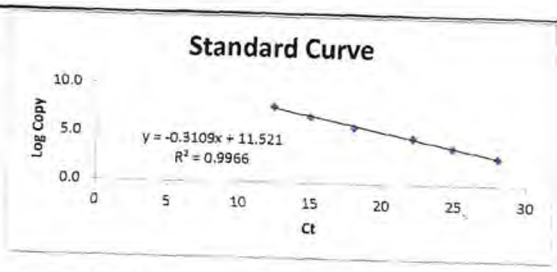
10/16/2017

Run Real-time PCR – iNOS with Standard & Samples

Gene information

Gene of Interest	iNOS		
		Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	89	bases	
Mass in Daltons	2.72E+04	Da	= number bases x avg. mass/base
Mass in grams	4.51E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	4.51E-14	ug	= above / 10E-6
Mass in ng	4.51E-11	ng/copy	= above x 10E3

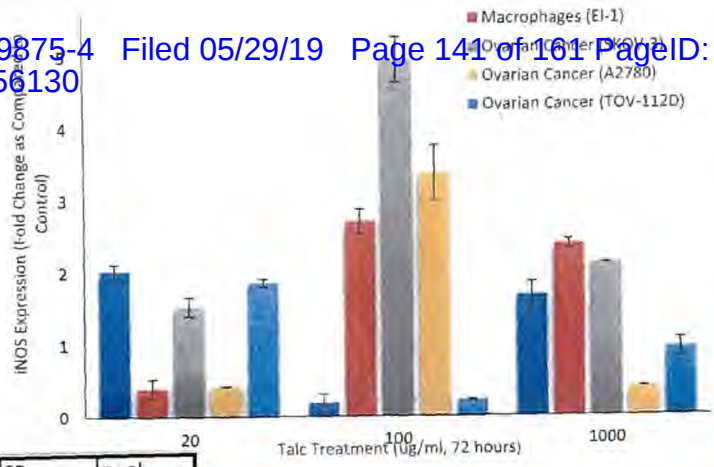
Copy #	Ct	Log Copy #
61500000	12.29	7.8
6150000	13.15	6.8
615000	16.12	5.8
61500	20.69	4.8
6150	24.74	3.8
615	28.15	2.8



Standard Curve

Normal Ov Epithelial Cells	fg/ul cDNA	Fold Change	Average	SD	p val
Normal Ov Epithelial -Control for 1000	0.024815	0.023762852			
	0.241742				
	0.022711				
Normal Ov Epithelial -Control for 20 100	0.126806	0.183761711			
	0.093339				
	0.240718				
Normal Ov Epithelial 20 ug/ml Talc	0.119802	4.041569265	2.018802	0.095183	0.0351
	0.070136	1.951497602			
	0.073335	2.086107216			
Normal Ov Epithelial 100 ug/ml Talc	0.234882	0.278189205	0.191452	0.122665	ns
	0.152123	-0.172171608			
	0.203004	0.10471476			
Normal Ov Epithelial 1000 ug/ml Talc	0.0606	1.550198328	1.677861	0.180542	0.065
	0.076926	2.237232983			
	0.066667	1.805523796			
EL-1 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
EL1 Control DMSO for 20 ug/ml	0.031913	0.032005997			
	0.033565				
	0.032099				
EL1 Control DMSO for 100 ug/ml	0.092988	0.109375796			
	0.131096				
	0.125764				
EL1 20 ug/ml Talc	0.041749	0.304399045	0.395871	0.129361	0.11
	0.047604	0.487343726			
	0.164327	4.134252728			
EL1 100 ug/ml Talc	0.392141	2.585260966	2.702807	0.166235	0.0135
	0.417854	2.820352453			
	0.029852	-0.72706732			
EL-1 1000 control	0.867264	0.946591901			
	0.881998				
	1.02592				
EL-1 1000 ug/ml Talc	3.243944	2.426971659	2.382584	0.062774	0.0135
	3.159909	2.338196032			

Data



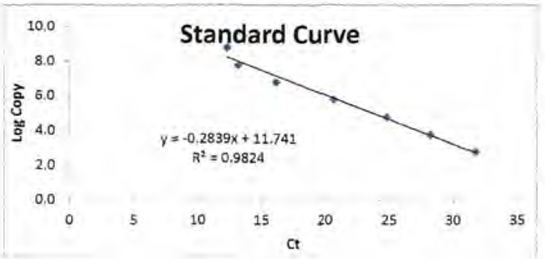
SKOV-3 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
SKOV control for 20 ug/ml Talc	0.018948	0.01936476			
	0.015705				
	0.019781				
SKOV-3 Control for 100 ug/ml Talc	0.013424	0.011345283			
	0.015051				
	0.009267				
SKOV-3 20 ug/ml	0.047144	1.434516565	1.52669	0.130353	0.0294
	0.050714	1.618863155			
	0.144431	6.458419298			
SKOV-3 100 ug/ml	0.06	4.288541523	4.949609	0.31163	0.05
	0.065	4.729253316			
	0.07	5.16996511			
SKOV-3 control for 1000 ug/ml Talc	1.369745	1.01247397			
	1.137957				
	0.655203				
SKOV-3 1000 ug/ml Talc	2.310336	1.281871867	2.117303	0.003559	0.0396
	3.15364	2.114786159			
	3.158736	2.119819883			
A2780 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
A2780 control for 20 ug/ml Talc	0.094243	0.079147127			
	0.068008				
	0.064051				
A2780 Control for 100 ug/ml Talc	0.053171	0.051076582			
	0.048683				
	0.048982				
A2780 20 ug/ml	0.112398	0.420119005	0.424255	0.005849	0.0629
	0.118215	0.493609261			
	0.113053	0.428390528			
A2780 100 ug/ml	0.209538	3.102432407	3.368828	0.37674	0.05
	0.180167	2.527397018			
	0.236751	3.635223784			
A2780 control for 1000 ug/ml Talc	4.549583	4.548883598			
	3.933995				
	4.548184				
A2780 1000 ug/ml Talc	6.369001	0.400123916	0.40196	0.002597	0.0032
	6.385709	0.403796873			
	6.86353	0.508838338			
TOV112 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	0.058522	0.052902734			
	0.058744				
	0.047283				
TOV112 Control 1000 volume	0.062537	0.064595234			
	0.068004				
	0.063245				
TOV112 20 ug/ml Talc	0.159626	2.017356105	1.854482	0.054431	0.0133
	0.148974	1.815992924			
	0.153046	1.892970354			
TOV112 100 ug/ml Talc	0.064349	0.216369215	0.222484	0.008648	0.0224
	0.064996	0.228599616			
	0.046148	-0.127677239			
TOV112 Control for 1000	0.053966	0.05318876			
	0.04459				
	0.06101				
TOV112D 1000 ug/ml Talc	0.078515	0.476157328	0.947326	0.121924	0.1077
	0.09899	0.861112589			
	0.108161	1.03353966			

10/16/2017 Run RT-PCR – GPX with standard & samples

Gene of Interest	GPX		
		Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	96	bases	
Mass in Daltons	2.93E+04	Da	= number bases x avg. mass/base
Mass in grams	4.86E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	4.86E-14	ug	= above / 10E-6
Mass in ng	4.86E-11	ng/copy	= above x 10E3

Gene information

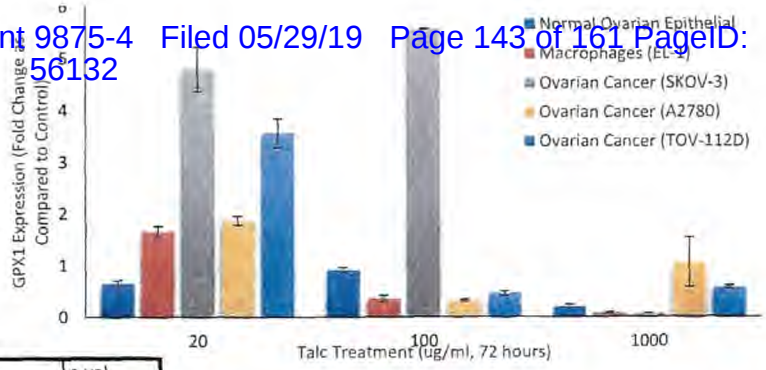
Copy #	Ct	Log Copy #
609000000	12.29	8.8
60900000	13.15	7.8
6090000	16.12	6.8
609000	20.69	5.8
60900	24.74	4.8
6090	28.15	3.8
609	31.71	2.8



Standard Curve

Data

Normal Ov Epithelial Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
Normal Ov Epithelial -Control for 1000	10.44452	12.39509			
	12.94154				
	13.79921				
Normal Ov Epithelial -Control for 20 100	12.52692	12.89282			
	13.25873				
	18.81577				
Normal Ov Epithelial 20 ug/ml Talc	25.05152	0.943059	0.640542	0.059069	0.0694
	21.68973	0.682311			
	20.61271	0.598774			
Normal Ov Epithelial 100 ug/ml Talc	24.93692	0.93417	0.902286	0.045092	0.0425
	24.11475	0.870401			
	21.4644	0.664833			
Normal Ov Epithelial 1000 ug/ml Talc	16.43495	0.325924	0.194533	0.042818	0.325
	15.18163	0.22481			
	14.43106	0.164256			
EL-1 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
EL1 Control DMSO for 20 ug/ml	26.43411	25.98751			
	24.94745				
	26.58098				
EL1 Control DMSO for 100/1000 ug/ml	26.2594	26.26166			
	26.03356				
	26.26393				
EL1 20 ug/ml Talc	66.79229	1.570168	1.644264	0.104787	0.0394
	70.6434	1.718359			
	77.07548	1.965866			
EL1 100 ug/ml Talc	36.51237	0.39033	0.345133	0.063918	0.0742
	46.80624	0.782303			
	34.13847	0.299935			
EL1 1000 regular	28.5	0.085232	0.075712	0.013463	0.0414
	28	0.066193			
	27	0.028115			



SKOV-3 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
SKOV control for 20 ug/ml Talc	6.817514	7.415123			
	7.716939				
	7.710917				
SKOV-3 Control for 100 ug/ml Talc	8.439988	8.412195			
	8.384401				
	8.053921				
SKOV-3 20 ug/ml	32.91981	3.439549	4.783855	0.421067	0.0315
	40.68022	4.486115			
	45.09577	5.081594			
SKOV-3 100 ug/ml	55.1	5.550015	5.555959	0.008406	0.0207
	52.1	5.19339			
	55.2	5.561902			
SKOV-3 control for 1000 ug/ml Talc	54.53351	52.15021			
	49.76691				
	39.85461				
SKOV-3 1000 ug/ml Talc	35.33473	-0.32244	0.070767	0.000524	ns
	55.82142	0.070397			
	55.86005	0.071138			
A2780 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
A2780 control for 20 ug/ml Talc	19.61021	20.77316			
	21.9361				
	16.44827				
A2780 Control for 100 ug/ml Talc	8.250935	8.317047			
	8.383159				
	6.165789				
A2780 20 ug/ml	23.21169	1.790857	1.854858	0.090511	0.0192
	24.27628	1.918858			
	21.72254	1.611809			
A2780 100 ug/ml	10.74368	0.291767	0.307519	0.022277	0.0162
	11.00571	0.323272			
	12.73299	0.530951			
A2780 control for 1000 ug/ml Talc	0.33411	0.215358			
	0.223734				
	0.206982				
A2780 1000 ug/ml Talc	0.368504	0.711124	1.047611	0.475865	0.291
	0.513434	1.384098			
	0.0282	-0.86906			
TOV112 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	15.60499	15.52105			
	16.04456				
	15.43712				
TOV112 Control 100 talc	5.87752	15.09463			
	14.00668				
	16.18258				
TOV112 20 ug/ml Talc	73.71172	3.749145	3.552248	0.278454	0.01
	67.59964	3.355352			
	59.39598	2.826801			
TOV112 100 ug/ml Talc	22.41454	0.484934	0.453197	0.044883	0.0465
	21.45642	0.42146			
	19.28461	0.27758			
TOV112 Control for 1000ug/ml Talc	4.893757	7.868342			
	7.055626				
	8.681058				
TOV112D 1000 Talc	14.70037	0.868293	0.56558	0.030965	0.1387
	12.49081	0.587476			

10/17/2017 Run RT-PCR – SOD3 with standard & samples

Gene information

Gene of Interest	SOD3	
		Unit
1 Dalton = 1.66E-24	1.66E-24	g
Mass of base pair	615	Da
Avg. Mass/base	305.25	Da
Length of entire	85	bases
Mass in Daltons	2.59E+04	Da = number bases x avg. mass/base
Mass in grams	4.31E-20	g = mass in Da x mass of a Da in grams
Mass in ug	4.31E-14	ug = above / 10E-6
Mass in ng	4.31E-11	ng/copy = above x 10E3

Copy #	Ct	Log Copy #
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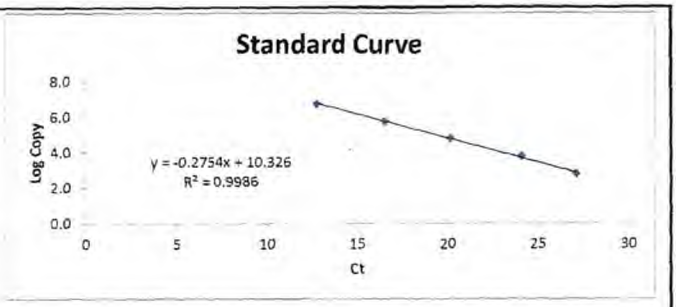
67000000	13.15	7.8
6099999.5	16.12	6.8
610000	20.69	5.8
61000	24.74	4.8
6100	28.15	3.8
610	31.71	2.8

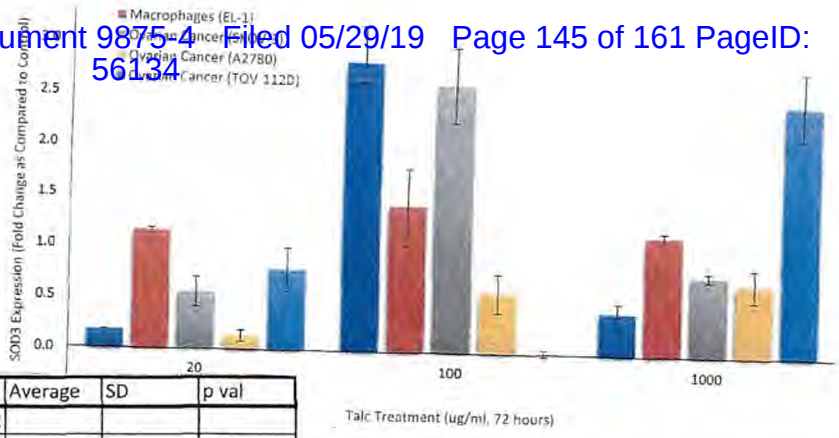


Standard Curve

Data

Normal Ov Epithelial Cells	fg/ul cDNA	Fold Change	Average	SD	p val
Normal Ov Epithelial -Control for 1000	0.069583	0.060875247			
	0.102165				
	0.052168				
Normal Ov Epithelial -Control for 200 500	1.025561	0.635120216			
	0.679916				
	0.590325				
Normal Ov Epithelial 20 ug/ml Talc	0.749187	0.17959921	0.179165	0.000614	0.2374
	0.748636	0.178730864			
	0.803527	0.265158094			
Normal Ov Epithelial 100 ug/ml Talc	3.517144	4.537760335	2.831552	0.347194	0.05
	2.277572	2.586048719			
	2.589421	3.077055842			
Normal Ov Epithelial 1000 ug/ml Talc	0.082194	0.350206214	0.4135	0.089512	0.05
	0.116134	0.907744026			
	0.0899	0.476794623			

EL-1 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
EL1 Control DMSO for 20 ug/ml	0.018405	0.01823182			
	0.017237				
	0.019054				
EL1 Control DMSO for 100/1000 ug/ml	0.110426	0.118911669			
	0.085674				
	0.127398				
EL1 20 ug/ml Talc	0.038627	1.118643876	1.137817	0.027115	0.0021
	0.0348	0.908736299			
	0.039326	1.156990307			
EL1 100 ug/ml Talc	0.254701	1.141934765	1.411269	0.380896	0.0887
	0.318755	1.680603474			
	0.380953	2.203659803			
EL1 1000 ug/ml Talc	0.26	1.186496936	1.147252	0.042327	0.0629
	0.25	1.1024009			
	0.256	1.152858521			



SKOV-3 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
SKOV control for 20 ug/ml Talc	0.006913	0.010297182			
	0.013535				
	0.010443				
SKOV-3 Control for 100 ug/ml Talc	0.012234	0.011557716			
	0.010882				
	0.050575				
SKOV-3 20 ug/ml	0.019103	0.652865731	0.551015	0.144039	0.05
	0.016749	0.449164129			
	0.022063	0.908963284			
SKOV-3 100 ug/ml	0.038926	2.367972796	2.626045	0.364969	0.05
	0.044892	2.884116792			
	0.026396	1.283859584			
SKOV-3 control for 1000 ug/ml Talc	0.208612	0.1401244			
	0.151128				
	0.129121				
SKOV-3 1000 ug/ml Talc	0.244017	0.741431658	0.770192	0.040674	0.04
	0.23448	0.673367285			
	0.252077	0.798953236			

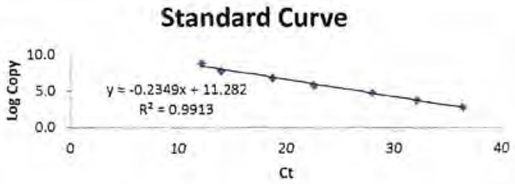
A2780 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
A2780 control for 20 ug/ml Talc	0.029232	0.021280566			
	0.028951				
	0.021281				
A2780 Control for 100 ug/ml Talc	0.039562	0.027201344			
	0.026435				
	0.027968				
A2780 20 ug/ml	0.028102	0.320560377	0.133381	0.055191	0.187
	0.024949	0.172406621			
	0.023288	0.09435509			
A2780 100 ug/ml	0.046663	0.715466188	0.584546	0.18515	0.1692
	0.039541	0.453625014			
	0.058702	1.15805571			
A2780 control for 1000 ug/ml Talc	0.052637	0.099050365			
	0.098587				
	0.099513				
A2780 1000 ug/ml Talc	0.178792	0.805058554	0.696194	0.153958	0.1029
	0.157225	0.587328489			
	0.068449	-0.308951611			

TOV112 Cells	fg/ul cDNA	Fold Change	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	0.030712	0.030110321			
	0.043831				
	0.029509				
TOV112 Control 100 talc	0.016775	0.014654626			
	0.016117				
	0.011072				
TOV112 20 ug/ml Talc	0.03435	1.343995658	0.77735	0.209132	0.1204
	0.028214	0.925229068			
	0.023879	0.629471258			
TOV112 100 ug/ml Talc	0.014484	-0.011613672	0.011113	0.032141	ns
	0.015151	0.033840165			
	0.018271	0.246757232			
TOV112 Control for 1000ug/ml Talc	0.031325	0.028505848			
	0.025687				
	0.068399				
TOV112D 1000 Talc	0.106165	2.724327168	2.490101	0.331245	0.05
	0.47479	15.65588928			

10/18/2017 Run RT-PCR CAT with standard & samples

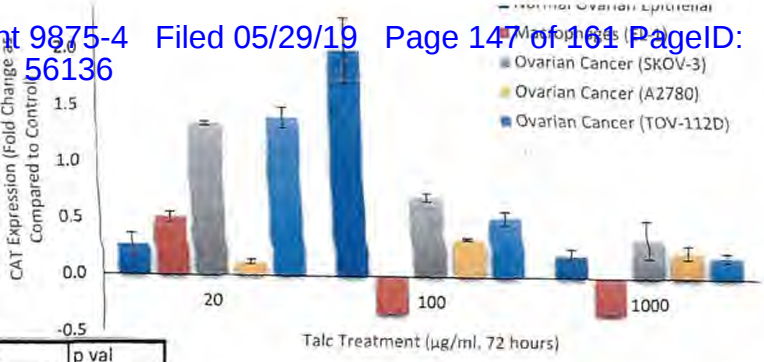
Gene of Interest	CAT		
		Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	105	bases	
Mass in Daltons	3.21E+04	Da	= number bases x avg. mass/base
Mass in grams	5.32E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	5.32E-14	ug	= above / 10E-6
Mass in ng	5.32E-11	ng/copy	= above x 10E3

Copy #	Ct	Log Copy #
606000000	12.29	8.8
60600000	13.15	7.8
6060000.5	16.12	6.8
606000	20.69	5.8
60600	24.74	4.8
6060	28.15	3.8
606	31.71	2.8



Normal Ov Epithelial Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
Normal Ov Epithelial -Control for 1000	0.255112	0.277963			
	0.300814				
Normal Ov Epithelial -Control for 200 500	0.275147	0.196178			
	0.264911				
	0.196178				
Normal Ov Epithelial 20 ug/ml Talc	0.23504	0.198092	0.266425	0.096638	0.161
	0.162371	-0.17233			
	0.261851	0.334759			
Normal Ov Epithelial 100 ug/ml Talc	0.629433	2.208474	2.006022	0.28631	0.05
	0.139599	-0.28841			
	0.55	1.80357			
Normal Ov Epithelial 1000 ug/ml Talc	0.263472	-0.05213	0.197083	0.057972	0.1312
	0.32135	0.15609			
	0.344139	0.238076			
EL-1 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
EL1 Control DMSO for 20 ug/ml	29.20198	28.39182			
	22.84908				
	27.58165				
EL1 Control DMSO for 100/1000 ug/ml	31.24367	30.90921			
	30.57474				
	33.16323				
EL1 20 ug/ml Talc	25.06856	-0.11705	0.51189	0.047031	0.0765
	41.98112	0.478634			
	43.86952	0.545146			
EL1 100 ug/ml Talc	20.73672	-0.32911	-0.33013	0.001441	0.0189
	17.25388	-0.44179			
	20.67373	-0.33115			
EL1 1000 ug/ml Talc	21	-0.32059	-0.31897	0.002288	0.0189
	21.1	-0.31736			
	22	-0.28824			

rdh



SKOV-3 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
SKOV control for 20 ug/ml Talc	2.474985	3.537794			
	3.399213				
	3.676375				
SKOV-3 Control for 100 ug/ml Talc	5.576323	5.393164			
	5.152521				
	5.450649				
SKOV-3 20 ug/ml	9.01118	1.547119	1.34984	0.017963	0.0245
	8.358183	1.362541			
	8.268313	1.337138			
SKOV-3 100 ug/ml	8.554721	0.586215	0.705385	0.035231	0.0462
	9.331777	0.730297			
	9.063065	0.680473			
SKOV-3 control for 1000 ug/ml Talc	14.71117	14.67545			
	14.63973				
	13.97333				
SKOV-3 1000 ug/ml Talc	15.39131	0.048779	0.347002	0.167419	0.2053
	21.50518	0.465385			
	18.03053	0.228619			

A2780 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
A2780 control for 20 ug/ml Talc	6.448358	5.673473			
	5.853449				
	5.493497				
A2780 Control for 100 ug/ml Talc	4.163294	3.876415			
	3.989297				
	3.763532				
A2780 20 ug/ml	7.589043	0.337636	0.121263	0.021465	0.2349
	6.275344	0.106085			
	6.447571	0.136442			
A2780 100 ug/ml	5.154843	0.329797	0.337465	0.010844	0.0691
	5.979143	0.542442			
	5.214293	0.345133			
A2780 control for 1000 ug/ml Talc	9.973024	9.842133			
	11.24123				
	8.312149				
A2780 1000 ug/ml Talc	11.69434	0.188192	0.230282	0.059524	ns
	12.52286	0.272372			
	16.00005	0.625669			

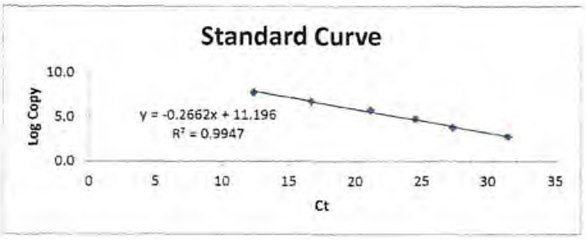
TOV112 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	3.382153	3.406415			
	3.150577				
	3.686515				
TOV112 Control 100 talc	0.838564	3.664997			
	3.735731				
	3.594263				
TOV112 20 ug/ml Talc	8.42336	1.472793	1.408291	0.091219	0.0134
	7.983921	1.34379			
	9.243696	1.713614			
TOV112 100 ug/ml Talc	2.319637	0.291634	0.528072	0.058693	0.0419
	2.818786	0.569574			
	2.66972	0.48657			
TOV112 Control for 1000ug/ml Talc	1.807987	1.795893			
	1.783799				
	2.391376				
TOV112D 1000 Talc	2.186972	0.217763	0.189448	0.040043	0.0722
	2.085271	0.161133			
	1.779704	-0.00901			

10/18/2017 Run RT-PCR MPO with Standard & Samples

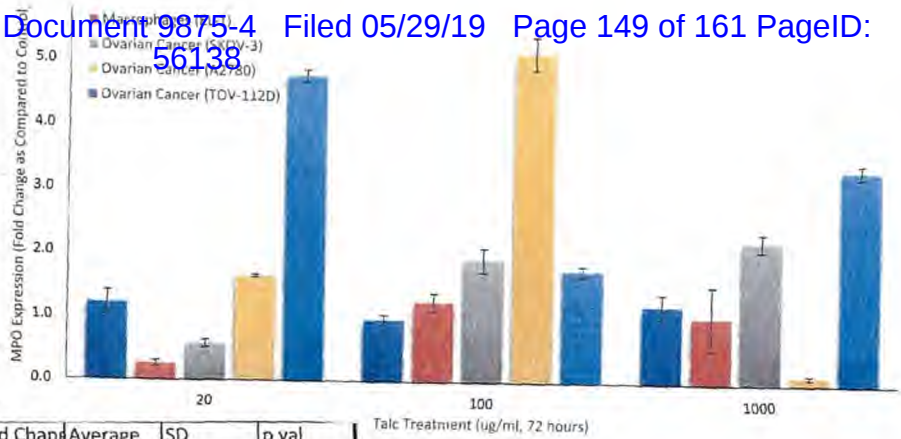
Gene of Interest	MPO		
		Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	79	bases	
Mass in Daltons	2.41E+04	Da	= number bases x avg. mass/base
Mass in grams	4.00E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	4.00E-14	ug	= above / 10E-6
Mass in ng	4.00E-11	ng/copy	= above x 10E3

Gene information

Copy #	Ct	Log Copy #
60900000	12.29	7.8
6090000	13.15	6.8
609000	16.12	5.8
60900	20.69	4.8
6090	24.74	3.8
609	28.15	2.8



Normal Ov Epithelial Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
Normal Ov Epithelial -Control for 1000	0.003502	0.003044			
	0.00298				
	0.003108				
Normal Ov Epithelial -Control for 200 500	0.003502	0.003044			
	0.00298				
	0.003108				
Normal Ov Epithelial 20 ug/ml Talc	0.006317	1.075409	1.206998	0.186096	0.05
	0.007118	1.338587			
	0.009902	2.253146			
Normal Ov Epithelial 100 ug/ml Talc	0.006142	1.017918	0.962795	0.077956	ns
	0.007321	1.405213			
	0.005807	0.907672			
Normal Ov Epithelial 1000 ug/ml Talc	0.006317	1.075409	1.206998	0.186096	0.05
	0.007118	1.338587			
	0.009902	2.253146			
EL-1 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
EL1 Control DMSO (5 ug/ml volume)	0.026276	0.025624			
	0.024419				
	0.026177				
EL1 20 ug/ml Talc	0.035331	0.37884	0.257395	0.044953	0.0242
	0.033034	0.289182			
	0.031405	0.225609			
EL1 100 ug/ml Talc	0.05	0.951307	1.244003	0.137978	0.0101
	0.055	1.146437			
	0.06	1.341568			
EL-1 1000 control	0.00479	0.004725			
	0.004184				
	0.005202				
EL-1 1000 ug/ml Talc	0.011248	1.380253	1.032749	0.491445	0.1629
	0.003024	-0.36008			
	0.007964	0.685245			



SKOV-3 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
SKOV control for 20 ug/ml Talc	0.022406	0.021915			
	0.021424				
	0.029877				
SKOV-3 Control for 100 ug/ml Talc	0.025145	0.016548			
	0.014775				
	0.018321				
SKOV-3 20 ug/ml	0.027	0.631639	0.571208	0.060431	0.1817
	0.025	0.510777			
	0.026	0.571208			
SKOV-3 100 ug/ml	0.045764	1.765596	1.897412	0.186416	0.0083
	0.050127	2.029228			
	0.028656	0.731711			
SKOV-3 control for 1000 ug/ml Talc	0.001542	0.001052			
	0.001059				
	0.001044				
SKOV-3 1000 ug/ml Talc	0.001403	0.333859	2.211632	0.136334	0.008
	0.003276	2.115229			
	0.003479	2.308034			

A2780 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
A2780 control for 20 ug/ml Talc	0.0108	0.01085			
	0.02				
	0.0109				
A2780 Control for 100 ug/ml Talc	0.063463	0.075407			
	0.072816				
	0.089943				
A2780 20 ug/ml	0.028626	1.638359	1.624942	0.018975	0.05
	0.026093	1.40486			
	0.028335	1.611525			
A2780 100 ug/ml	0.064579	4.951951	5.130818	0.252956	0.05
	0.056682	4.224148			
	0.06846	5.309685			
A2780 control for 1000 ug/ml Talc	0.004955	0.004432			
	0.004108				
	0.004233				
A2780 1000 ug/ml Talc	0.004912	0.108294	0.129488	0.029972	0.5279
	0.005751	0.297523			
	0.0051	0.150681			

TOV112 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	0.088068	0.075135			
	0.070801				
	0.066536				
TOV112 Control 100 talc	0.10722	0.108482			
	0.097003				
	0.121222				
TOV112 20 ug/ml Talc	0.042682	5.583981	4.748687	0.092276	0.05
	0.036844	4.683438			
	0.03769	4.813937			
TOV112 100 ug/ml Talc	0.019449	2.000104	1.742775	0.075626	0.05
	0.017434	1.689299			
	0.018127	1.79625			
TOV112 Control for 1000ug/ml Talc	0.008193	0.006483			
	0.005154				
	0.0061				
TOV112D 1000 Talc	0.014068	1.170051	3.334727	0.107619	0.05
	0.027607	3.258629			

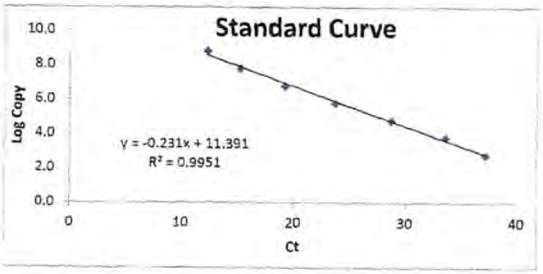
10/19/2017 Run RT-PCR GSTp1 with Standard & samples

Gene of Interest	GSTp1		
		Unit	Formula
1 Dalton = 1.66E-24	1.66E-24	g	
Mass of base pair	615	Da	
Avg. Mass/base	305.25	Da	
Length of entire	100	bases	
Mass in Daltons	3.05E+04	Da	= number bases x avg. mass/base
Mass in grams	5.07E-20	g	= mass in Da x mass of a Da in grams
Mass in ug	5.07E-14	ug	= above / 10E-6
Mass in ng	5.07E-11	ng/copy	= above x 10E3

Gene information

Standard Curve

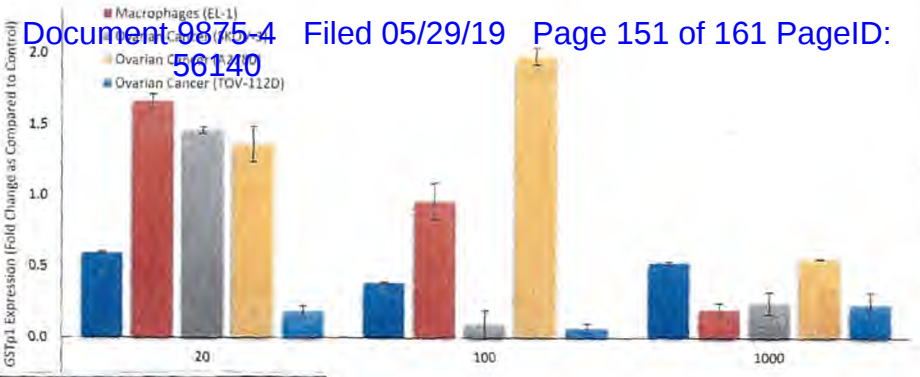
Copy #	Ct	Log Copy #
606000000	12.29	8.8
60600000	13.15	7.8
6060000.5	16.12	6.8
606000	20.69	5.8
60600	24.74	4.8
6060	28.15	3.8
606	31.71	2.8



Data

Normal Ov Epithelial Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
Normal Ov Epithelial -Control for 1000	4.5	4.42			
	4.4				
	4.44				
Normal Ov Epithelial -Control for 200 500	4.5	4.42			
	4.4				
	4.44				
Normal Ov Epithelial 20 ug/ml Talc	7	0.58371	0.592006	0.007273	0.003
	7.05	0.595023			
	7.06	0.597285			
Normal Ov Epithelial 100 ug/ml Talc	6.1	0.38009	0.385747	0.007999	0.004
	6.05	0.368778			
	6.15	0.391403			
Normal Ov Epithelial 1000 ug/ml Talc	6.8	0.538462	0.527149	0.011312	0.05
	6.7	0.515837			
	6.75	0.527149			

EL-1 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
EL1 Control DMSO (5 ug/ml volume)	10.20286	10.36977			
	9.75591				
	11.15053				
EL1 Control DMSO (1000 ug/ml volume)	34.79645	33.68753			
	32.57861				
	68.16306				
EL1 20 ug/ml Talc	24.71735	1.383597	1.665897	0.049681	0.0051
	28.00903	1.701027			
	27.28045	1.630768			
EL1 100 ug/ml Talc	19.38792	0.869658	0.959908	0.127633	0.0711
	21.25967	1.050158			
	33.05055	2.187203			
EL1 1000 ug/ml Talc	41.59777	0.234812	0.201666	0.046876	0.0007
	39.36456	0.16852			
	33.1334	-0.01645			



SKOV-3 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
SKOV control for 20 ug/ml Talc	36.31595	35.95677			
	35.5976				
	68.59786				
SKOV-3 Control for 100 ug/ml Talc	72.75467	61.72977			
	58.02745				
	65.4321				
SKOV-3 20 ug/ml	87.92087	1.445182	1.461944	0.023705	0.0116
	89.12626	1.478706			
	84.90128	1.361204			
SKOV-3 100 ug/ml	71.91819	0.165049	0.09506	0.098979	ns
	63.27742	0.025071			
	32.76049	-0.46929			
SKOV-3 control for 1000 ug/ml Talc	4.842595	4.837675			
	6.244395				
	4.832755				
SKOV-3 1000 ug/ml Talc	5.783046	0.195418	0.249358	0.076282	0.1381
	3.650555	-0.24539			
	6.304928	0.303297			

A2780 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
A2780 control for 20 ug/ml Talc	59.49055	30.29235			
	34.42553				
	26.15917				
A2780 Control for 100 ug/ml Talc	12.54541	13.77486			
	13.56743				
	13.9823				
A2780 20 ug/ml	31.33308	1.274657	1.362953	0.12487	0.0342
	33.76562	1.451249			
	25.47339	0.849267			
A2780 100 ug/ml	9.139274	1.941376	1.983008	0.058877	0.05
	9.397987	2.02464			
	7.876923	1.535102			
A2780 control for 1000 ug/ml Talc	2.663943	3.107142			
	3.176421				
	3.481062				
A2780 1000 ug/ml Talc	4.842595	0.558537	0.556953	0.002239	0.0661
	6.244395	1.009691			
	4.832755	0.55537			

TOV112 Cells	fg/ul cDNA	Fold Chang	Average	SD	p val
TOV112 Control for 20 ug/ml Talc	20.39414	20.7106			
	16.15828				
	21.02706				
TOV112 Control 100 talc	20.17126	19.40386			
	22.03957				
	18.63647				
TOV112 20 ug/ml Talc	27.94732	0.349421	0.186779	0.037984	0.0394
	24.02265	0.15992			
	25.13518	0.213638			
TOV112 100 ug/ml Talc	21.15492	0.090243	0.068337	0.030979	ns
	16.15982	-0.16719			
	20.30481	0.046431			
TOV112 Control for 1000ug/ml Talc	5.996679	6.766235			
	7.535791				
	9.979309				
TOV112D 1000 Talc	10.08078	0.489866	0.234578	0.089358	ns
	8.780969	0.297763			

School of Medicine

Talcum Powder Enhances Oxidative Stress in Ovarian Cancer Cells

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BACKGROUND

- We have previously characterized epithelial ovarian cancer (EOC) cells to manifest a persistent pro-oxidant state as evident by the upregulation of certain key oxidant and downregulation of key antioxidant enzymes.
  - This redox state is further enhanced in chemoresistant EOC cells.
- Several studies have suggested a possible association between genital use of talcum powder and risk of EOC; however, the biologic basis for this association has yet to be delineated.

OBJECTIVE

To determine the effects of talcum powder on the expression of key oxidant and antioxidant enzymes in EOC cells.

METHODS

- **Cell Culture:** Human ovarian cancer cell lines, SKOV-3 (HTB-77) and TOV-112D (CRL-11731), as well as human macrophages (EL-1, CRL-9854) were all obtained from American Type Culture Collection (ATCC). The ovarian cancer cell line A2780 was obtained from Sigma Aldrich. Human primary ovarian surface epithelium cells from Cell Biologics. Cells were seeded in 60mm² culture dishes (1.0×10^6) and allowed to rest for 24 hours.
- **Cell Treatment:** Talcum powder was obtained from Sigma Aldrich and was prepared in DMSO. Cell lines were treated with talcum powder (0, 20, 100, 1000 µg/ml) for 72 hours. Additionally, talc was soaked in DMSO for 72 hours, spun down, and supernatant collected and was used to treat cells (1000 µg/ml, referred to as "supernatant").
- **Real-time RT-PCR Analysis:** Total RNA was isolated from cells utilizing a RNeasy Extraction Kit (Qiagen). cDNA synthesis was performed using the SuperScript VILO Master Mix Kit (Life Technologies). Quantitative real-time RT-PCR was performed using a QuantiTect SYBR Green RT-PCR kit (Qiagen) and a Cepheid 1.2f Detection System. A standard with a known concentration was designed specifically for β-actin, MPO, iNOS, CAT, SOD3, GSR, GPX, GSTp1 using the Beacon Designer software. This allowed for absolute quantification of gene expression as copy numbers per microgram of RNA. Following real-time RT-PCR, a melting curve analysis was performed to demonstrate the specificity of the PCR product as a single peak. All samples were normalized to β-actin. A control, which contained all the reaction components except for the template, was included in all experiments.
- **Statistical Analysis:** Data were analyzed using SPSS 23.0 for Windows. Data was analyzed with one way ANOVA followed by Tukey's post hoc tests with Bonferroni correction.

RESULTS

There was a marked increase in mRNA levels of the pro-oxidant enzymes, iNOS and MPO in talc treated ovarian cancer cell line, macrophages, and normal ovarian epithelial cells, all as compared to their control (Figure 1A&B). Additionally, there was a marked increase in the mRNA levels of the antioxidant enzymes CAT, SOD3, GSR, GPX1 and GSTp1, in talc treated ovarian cancer cell lines and in normal ovarian epithelial cells, all compared to their control (Figures 1&2). Interestingly, macrophages had decreased CAT mRNA levels at the 100, 1000, and supernatant doses (Figure 2D).

RESULTS

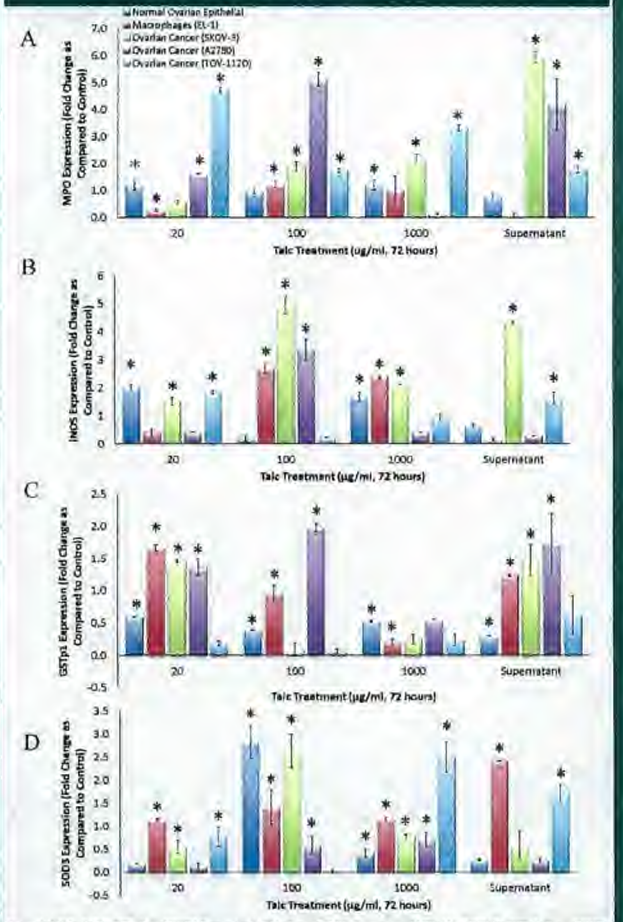

Figure 1: MPO, iNOS, GSTp1, and SOD3 Expression in Talc Treated Cells. Expression of MPO (A), iNOS (B), GSTp1 (C), and SOD3 (D) mRNA levels in normal ovarian epithelial cells, macrophages, and ovarian cancer cell lines (SKOV-3, A2780, and TOV-112D) after treatment with talc (20, 100, 1000, and supernatant from 1000 µg/ml) for 72 hours was determined by real-time RT-PCR. Fold change was calculated as compared to control. *P<.05 vs. controls.

RESULTS

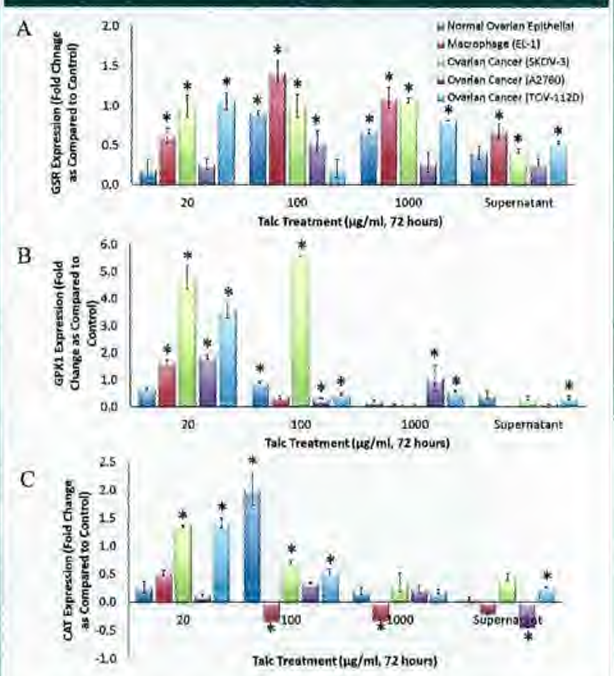

Figure 2: GSR, GPX1, and CAT Expression in Talc Treated Cells. Expression of GSR (A), GPX1 (B), SOD3 (C) and CAT (D) mRNA levels in normal ovarian epithelial cells, macrophages, and ovarian cancer cell lines (SKOV-3, A2780, and TOV-112D) after treatment with talc (20, 100, 1000, and supernatant from 1000 µg/ml) for 72 hours was determined by real-time RT-PCR. Fold change was calculated as compared to control. *P<.05 vs. controls.

CONCLUSIONS

This is the first report to show that talcum powder induces a biological effect by further enhancing the redox state in normal macrophages and ovarian epithelial cells as well as in ovarian cancer cells. The results of this study will provide a molecular basis to previous reports that link genital use of talcum powder to increased risk of epithelial ovarian cancer.

Exhibit J

two latent change-point states within VENG: one of high change-point frequency, and one of low change-point frequency indicated an underlying structure in fetal and neonatal VENG.
CONCLUSION: HRV monitoring tracks fetal and neonatal inflammatory response using abdominal ECG or regular precordial ECG, respectively. VNS reduces the magnitude of the neonatal inflammatory response. The proposed VENG analysis can provide insights into dynamics of vagal signalling to optimize the anti-inflammatory VNS regimens. This is clinically relevant because VNS can be done non-invasively.
*Figure(s) will be available online.

F-096

Use of Songs to Improve Knowledge of Antenatal Care in a Predominantly Illiterate Community. Binod B Sharma,[1] Deborah Loxton*,[1] Henry Murray,[2] Giavanna Angeli,[2] Simon Chiu,[2] Christopher Oldmeadow,[1] Roger Smith*,[3] [1]Hunter Medical Research Institute (HMRI), New Lambton, Australia; [2]Hunter Medical Research Institute HMRI, New Lambton, Australia; [3]John Hunter Hospital, New Lambton, Australia; [4]University of Newcastle, Newcastle, Australia; [5]University of Newcastle Australia, New Lambton, Australia.
INTRODUCTION: In many rural villages of Nepal, the maternal mortality rate is amongst the highest in the world. The reasons for this are cultural, illiteracy and lack of knowledge about the needs of women during pregnancy. The death of a woman in childbirth is just accepted. Culturally, singing and dancing are part of Nepalese daily life in rural settings. We hypothesized that health messages regarding the importance of antenatal care might be effectively transmitted by songs in the limited literacy environment of rural Nepal.
METHODS: We randomly grouped four rural Village Development Committees comprising 36 villages into two (intervention and control) clusters. In the intervention group, community members were provided with key health messages regarding pregnancy and childbirth, and different local groups were invited to write song lyrics incorporating the messages to accompany locally popular melodies. The local groups presented their songs and dance in a community festival organised and judged by the community. The winning songs were performed by the local people in a song and dance progression through the villages, houses and fields. A wall chart with key health messages was also provided to each household. Knowledge of household decision makers (senior men and women) was assessed before and after the intervention using a structured questionnaire in all households. Each stage of the process was video recorded.
RESULTS: Baseline and post-intervention survey (intervention n=735, control n=775) data was collected. Knowledge scores were evaluated as the number of correct items out of 36 questions. Knowledge improved significantly in the intervention group, improving from a mean of 11·60/36 to 22·33/36, an increase of 10·69 [9·97, 11·41, P<0·001]. The control population improved from 17·48/36 to 18·26/36 a mean increase of 0·81 units [0·28, 1·33]. Improvement was greatest amongst the most illiterate members of the community [6.8, 19.8, P<0·001]. No changes were observed in the control villages.
CONCLUSION: The use of singing bypassed the limitations of literacy in communicating health messages that are key to improving maternal mortality in this rural setting within a developing country. With appropriate sociocultural adaptation to local context, this model of community education may be applicable to improve maternal health outcomes in other low resource communities.

F-097

Stromal Cell Expression of the Receptor Tyrosine Kinase DDR2 Promotes Ovarian Cancer Metastasis. Katherine Fuh, Molly Greenwade, Whitney Grither, Hollie Beck, Daniel Wilke, Ian Hagemann, Andrea Hagemann, Carolyn McCourt, Premal Thaker, Matt Powell, Dave Mutch, Greg Longmore. *Washington University, St. Louis, MO, United States.*
INTRODUCTION: Understand the role of stromal discoid domain receptor 2 (DDR2) expression in ovarian cancer metastasis
METHODS: Immunohistochemistry was performed of short-term (<3 years) versus long-term (>5 years) high-grade serous ovarian cancer tumors through the Washington University Gynecologic Oncology Tissue bank. Stromal and tumor cell expression of DDR2 was scored for intensity and frequency. To determine the effect of metastasis with DDR2 deficient stromal cells, global DDR2 knockout (KO) mice (DDR2 deficient) were compared to DDR2 wild-type (WT) mice (DDR2 expresing) when a DDR2 expressing murine tumor cell line (ID8Trp53-/-BRCA2-/-) was injected intraperitoneally. Intraperitoneal spread was quantified using bioluminescence imaging (BLI) and tumor weight. Additionally, cell based mesothelial cell clearance assays utilizing human omentum-cultured mesothelial cells expressing DDR2 were compared to mesothelial cells not expressing DDR2.
RESULTS: Patients who lived <3 years had significantly higher DDR2 expression in the stroma when compared to patients living >5 years (mean DDR2 IHC score 76% vs 48%, p<0.0001). Similar findings were observed for DDR2 expression in the tumor cells, with mean IHC score 80% vs 64%, p<0.0001 in patients who lived <3 years vs >5 years. In determining the stromal effect of DDR2 in metastasis, DDR2 KO mice (DDR2 deficient in stromal cells) had less intraperitoneal spread of ovarian cancer cells than DDR2 WT by BLI (mean 5.8×10^8 vs 2.2×10^9 total photon flux , p=0.01) and by number of tumor implants (mean 6 vs 2 nodules, p=0.006). Additionally, human ovarian cancer cells plated above mesothelial cells that were DDR2 deficient had less tumor cell clearance than those tumor cells plated above mesothelial cells that expressed DDR2 (p=0.01).
CONCLUSION: The stromal contribution of DDR2 promotes tumor cell clearance of mesothelial cells and metastatic spread. This suggests that stromal expression of DDR2 may be a potential target to guide future therapy particularly in the maintenance setting.

F-098

Talcum Powder Enhances Oxidative Stress in Ovarian Cancer Cells. Nicole M Fletcher*, Ira Memaj, Ghassan M Saed*. *Wayne State University, Detroit, MI, United States.*
INTRODUCTION: We have previously characterized epithelial ovarian cancer (EOC) cells to manifest a persistent pro-oxidant state as evident by the upregulation of certain key oxidant and downregulation of key antioxidant enzymes. This redox state is further enhanced in chemoresistant EOC cells. Several studies have suggested possible association between genital use of talcum powder and risk of EOC, however, the biologic basis for this association has yet to be delineated. The objective of this study was to determine the effects of talcum powder on the expression of key oxidant and antioxidant enzymes in EOC cells.
METHODS: Human EOC cell lines (SKOV-3, MDAH-2774, A2780, and OV90) were obtained from ATCC and Sigma Aldrich. Human primary normal ovarian epithelial cells were obtained from Cell Biologics. Cells were treated with 0, 200, 500 µg/ml of talc (Sigma Aldrich) for 24, 48, and 72 hrs. RNA was extracted, followed by cDNA synthesis and real-time RT-PCR was performed to determine mRNA levels of key redox enzymes including myeloperoxidase (MPO), inducible nitric oxide synthase (iNOS), superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GPX), and glutathione reductase (GSR). Data was analyzed with oneway ANOVA. Significant comparisons were further analyzed with Tukey's post hoc tests with Bonferroni correction.
RESULTS: There was a marked increase in mRNA levels of the pro-oxidant enzymes, iNOS and MPO in talc treated ovarian cancer cell lines and normal ovarian epithelial cells, all as compared to their control, as early as 24 hours in all doses. Additionally, there was a marked decrease in the mRNA levels of the antioxidant enzymes CAT, GPX, SOD3, but with a marked increase in GSR, and no change in GST, in talc treated ovarian cancer cell lines and normal ovarian epithelial cells, all as compared to their control, as early as 24 hours in all doses.
CONCLUSION: This is the first report to show that talcum powder induces a biological effect by further enhancing the redox state in both normal ovarian epithelial cells as well as in ovarian cancer cells. The

results of this study will provide a molecular basis to previous reports that link genital use of talcum powder to increased risk of epithelial ovarian cancer.

F-099

ERAP2(N) Induced Rapid Choriocarcinoma Clearance *In Vivo*. Eun D Lee*,[3] Michelle Warthan,[2] Sonya Washington,[3] Ronald Ramus,[3] Efstratios Stratikos,[4] Jerome Strauss.[3] [1]*National Center for Scientific Research, Demokritos, Greece;* [2]*University of Virginia, Charlottesville, VA, United States;* [3]*Virginia Commonwealth University, Richmond, VA, United States.*

INTRODUCTION: High as 50 percent of hydatidiform mole results in life threatening gestational choriocarcinoma when the tumor metastases. Half of the choriocarcinoma cell lines lack endoplasmic reticulum aminopeptidase 2 (ERAP2) and have a unique HLA repertoire to study the immune mechanism. ERAP2 enzyme trims amino acid residues prior to presentation on HLA class I molecules. When the major T allele of ERAP2 changes Lysine (K) to asparagine (N) near the catalytic center of the enzyme it results in increased peptide trimming by up to 165-fold. This alters the peptide and HLA repertoire affecting the immune response. Interestingly, ERAP2(N) is not biologically detected in any population studied. Therefore, we hypothesize that ERAP2(N) can induce fatal immune response. Using an ERAP2(N) expressing choriocarcinoma cell model, our preliminary data shows that ERAP2(N) expressing cells are preferentially killed by activated NK cells *in vitro*. This observation suggests that ERAP2(N) expression in cells is immunologically unfavorable for survival.

METHODS: To further test the role of ERAP2(N) *in vivo*, we used NSG mice model system to determine its contribution to the emergence and clearance of solid tumors by adoptive transfer of immune cells compared to the tumor that does not express ERAP2(N).

RESULTS: After the lymphocyte treatment, only ERAP2(N) tumor displayed a rapid and significant decrease in tumor volume (P=0.046). The DiR fluorescently labeled lymphocytes were specifically targeting the tumor. The TdT Immunohistochemistry analysis of the tumor confirmed the apoptotic death by the activated lymphocyte. Lastly, the activated lymphocytes were elevated against ERAP2(N) expressing choriocarcinoma cells.

CONCLUSION: Together, the data strongly suggest that ERAP2(N) can be utilized as a potential cancer target molecule to specifically eliminate tumor.

F-100

Trends amongst Residency Programs Offering Trials of Labor after Cesarean Delivery (TOLAC). Monica Basinger†, Jerasimos Ballas*, Christina Davidson*. *Baylor College of Medicine, Houston, TX, United States.*

INTRODUCTION: The National Institutes of Health recognizes that a trial of labor after cesarean delivery (TOLAC) is a reasonable option for many women with a prior cesarean delivery and called on organizations to facilitate access to this birth option. However, the practice of offering TOLAC varies widely throughout the United States. The purpose of our study is to determine what trends exist in the attitudes and practices of offering TOLAC between different levels of residents and Program Directors, different types of U.S. OB/GYN Residency training programs, and different regions of the country.

METHODS: A voluntary survey was sent electronically via publicly acquired e-mail addresses of Program Directors to each current U.S. OB/GYN ACGME accredited program with request to complete survey and distribute amongst their residents.

RESULTS: We had 30% (80/249) response rate from program directors and estimated 4.8% (243/5020) total resident responses, assuming every resident was distributed the survey. The majority of responses were from the Mid-Atlantic (20%) and South Atlantic (16-19%) regions. Program types were divided into four categories with the majority of responses from University based programs (60%) and Community hospital, university-affiliated (27%) followed by community based (12%) and military (1%). Both program directors and residents answered similarly in that 100% of both groups reported their program offered TOLAC to patients with one prior cesarean, 81% offer TOLAC with two prior cesareans and 10% offer TOLAC in patients with three prior cesareans. Using chi-squared analysis, there was no statistically significant difference in offering TOLAC to different candidates in both the type of hospital and the region of the county.

In general, both groups felt the attitude of hospital nursing staff towards TOLAC was positive. The vast majority of program directors felt comfortable with their residents managing and counseling patients regarding TOLAC (99%). Likewise, most residents responded affirmatively that they are comfortable with the intrapartum management of a woman undergoing TOLAC (95%) and 82% agree that post-residency, they will offer TOLAC to appropriate candidates regardless of indication for cesarean.

CONCLUSION: Program directors and residents responded similarly in the rates of offering TOLAC to certain patients depending on risk factors. Both groups also felt confident in the ability of residents to manage these patients in labor. There were no significant differences in programs who offered TOLAC based on type of program or region of the country. Specifically, the Mid-Atlantic region (NY, NJ, and PA) was not associated with a decrease in the number of patients being offered TOLAC compared to all other regions despite these states being reported as three of the top five highest for medical malpractice payouts per capita in 2016.

F-101

Assessment of Fetal Head Position and Primary Cesarean Delivery Rate. Chelsea DeBolt†,[1] Maya Craffey†,[1] David O'Sullivan*,[1] Jessica Mullins*,[1] Adam Borgida*.[1] [1]*Hartford Hospital, Hartford, CT, United States;* [2]*University of Connecticut, Farmington, CT, United States.*

INTRODUCTION: Occiput posterior (OP) fetal head position is associated with higher rates of cesarean delivery (CD), prolonged second stage of labor, operative vaginal delivery (OVD) and third- and fourth-degree perineal lacerations. Despite national concern for higher than recommended CD rate, few interventions have been shown to decrease the CD rate overall. The objective of this study is to evaluate if assessment of the fetal head position beyond 6 cm dilation but prior to the start of the second stage of labor is associated with a decrease in primary CD rate.

METHODS: This retrospective cohort study included nulliparous, term, singleton, vertex pregnancies delivered at Hartford Hospital from August 2016 to April 2017. The exposure group consists of patients who had assessment of fetal head position, while the control group consists of patients who did not. The primary outcome was CD rate. Groups were compared with respect to each maternal and neonatal variable and differences between groups were evaluated with a Pearson chi square test. A multivariate regression model was performed to evaluate the influence of several variables on the outcome. All data was analyzed using an a priori alpha level of 0.05.

RESULTS: 690 women met inclusion criteria for investigation, 379 women in the control group and 311 women in the exposure group. The primary CD rate was 15.9% in the control group and 22.9% in the exposure group, and this difference was not statistically significant (p=0.078, Table 1). Arrest of the second stage of labor was more likely to be the reason for CD in the exposure group (53.4% vs. 19.2%, p<0.001, Table 1), and within the second stage arrest group, persistent OP occurred in 58.1% of deliveries. The OVD rate was also higher in the exposure group (10.0% vs. 5.8%, p=0.041, Table 1). For those who underwent an intervention (i.e. manual rotation, position changes) within the exposure group, the rate of CD was significantly higher (42.5% vs. 22.1%, p<0.001).

Friday Posters

Exhibit K

Society of Reproductive Investigation (SRI)

65th Annual Scientific Meeting, March 6-10, 2018

General Abstract Information

Abstract submission is now closed.

Abstract Submission Guidelines

- A non-refundable processing fee of $40.00 (US) is required for each abstract. Payment must be completed online using Visa, Mastercard, Discover, or American Express to complete the submission process.
- You do not need to be a member of SRI to submit an abstract.
- Three (3) Keywords for each abstract will be included.
- You are allotted 2,700 characters, which will include your Abstract Title, Abstract Body, Authors/Institutions, Tables and Figures.
- Please review the SRI Policy on Abstract Withdrawal and confirm compliance on the Questionnaire page
- If accepted for presentation at the meeting, the abstract will be published as entered into the 2018 Annual Meeting Program. However, the Editors reserve the right to edit any abstract that contains grammatical errors.
- Each submitted abstract will be reviewed by 4-6 expert reviewers, scored according to criteria described below, and 15% of the accepted abstracts will be chosen for oral presentation. Please note that as the SRI Program Committee is committed to maintaining high standards for the meeting, it is anticipated that a number of low-ranking abstracts will be rejected following peer-review.
- Case Reports are not generally encouraged for submission as abstracts unless they significantly advance the field of Reproductive Science.
- **The abstract receipt deadline was extended to October 20, 2017, 11:59 PM, Central Standard Time. This will remain firm and any abstracts received after the deadline will not be accepted.**

Evaluation of abstracts will be based on the following considerations:

a. **Originality/Innovation:** Is the idea or approach novel or is the work primarily confirmatory and/or a direct extension of previous work?
b. **Significance/Relevance:** Does the work address an important problem?
c. **Objective/Hypothesis:** Is an objective, specific research question and/or hypothesis stated?
d. **Methods:** Are the methods described? Are the methods employed appropriate to the research question? Were new methods used and validated?
e. **Results:** Are the results described clearly and succinctly? Was data evaluated statistically?
f. **Discussion:** Is the conclusion supported by the data

Click here to view examples of excellent abstracts from the 2016 Annual Scientific Meeting.

Changes or Edits to Abstracts: New in 2018!

SRI is using an upgraded system which now allows abstract submitters to make changes to their abstract on their own, up until the **extended deadline of October 22, 2017 at 11:59 PM CST.** However, if edits must occur after this date, an email must be sent to sri@support.ctimeetingtech.com

If you must withdraw your abstract, please be sure to review the SRI Policy on Abstract Withdrawal to confirm compliance before requesting a withdrawal.

Previously Presented Abstracts

- Abstracts cannot contain data previously presented at a national or international meeting at the time of abstract submission.
- Abstracts cannot contain data already accepted for publication in any online or PubMed indexed venue at the time of abstract submission.
- Violators will be subject to abstract withdrawal, and may be barred from presenting at future SRI meetings.

Abstract Notifications

Abstract notifications will be sent by email from CTI Meeting Technology, the abstract processor for SRI, in late December 2017. Contact authors should add @support.ctimeetingtech.com to their safe senders list to ensure they receive the email.

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Exhibit L

Session Late Breaking Poster Session

O Add To Itinerary

LB-044 - Talcum Powder Enhances Cancer Antigen 125 Levels in Ovarian Cancer Cells and in Normal Ovarian Epithelial Cells

March 10, 2018, 9:30 AM - 11:30 AM | Sapphire C - P

Categories
6.0 - Gynecologic Oncology

Keywords
CA-125,talc,epithelial ovarian cancer

Authors
Nicole M. Fletcher, Ph.D., Ghassan M. Saed, Ph.D..
Wayne State University, Detroit, MI, USA.

Abstract

Introduction: We have previously characterized epithelial ovarian cancer (EOC) cells to manifest a persistent pro-oxidant state as evident by the upregulation of certain key oxidant and downregulation of key antioxidant enzymes. Cancer antigen 125 (CA-125) is a protein produced on the surface of cells which is released into the blood stream. It is currently approved by the FDA to monitor the effectiveness of treatment for ovarian cancer and for detecting disease recurrence after treatment. CA-125 levels are elevated in more than 80% of women with advanced ovarian cancers, and in 50% of women with early stage cancers however, it's also elevated in benign conditions, such as diverticulitis, endometriosis, liver cirrhosis, pregnancy, and uterine fibroids. Several studies have suggested a possible association between genital use of talcum powder and risk of EOC; however, the biologic basis for this association has yet to be delineated. The objective of this study was to determine the effects of talcum powder on the CA-125 levels in EOC cells and normal ovarian epithelial cells.

Methods: EOC cell lines (OV-90 and TOV-21G) were obtained from ATCC. Human primary normal ovarian epithelial cells were obtained from Cell Biologics. Cells were treated with or without 1000 µg/ml of talc (Sigma Aldrich) for 72 hrs. Levels of CA-125 were determined in cell culture media using an ELISA. Data was analyzed with paired t-tests.

Results: There was a 1.4 ± 0.5 and 4.4 ± 0.5 fold increase in CA-125 levels in the talc treated OV90 and TOV-21G EOC cell lines, respectively, as compared to control. Similarly, there was a 1.7 ± 0.5 fold increase in CA-125 levels in normal ovarian epithelial cells as compared to control.

Conclusion: Talcum powder induces a biological effect by further enhancing CA-125 levels in ovarian cancer cells as well as in normal ovarian epithelial cells. This will provide a molecular basis to previous reports that link genital use of talcum powder to increased risk of epithelial ovarian cancer.